FAR EASTERN ASSOCIATION OF TROPICAL MEDICINE

TRANSACTIONS OF THE SEVENTH CONGRESS.

FAR EASTERN ASSOCIATION OF TROPICAL MEDICINE

TRANSACTIONS

OF THE

SEVENTH CONGRESS

HELD IN

BRITISH INDIA

DECEMBER, 1927

EDITED BY

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Director Pasteur Institute of India

Kasauli British India,

General Organizing Secretary for the Seventh Congress

VOLUME II.



Published for

THE SEVENTH CONGRESS

By

THACKERS PRESS & DIRECTORIES LTD

6 Mangoe Lane CALCUTTA

Printed by
THACKER'S PRESS & DIRECTORIES LTD
6 Mangoe Lane Calcutta

CONTENTS

(Volume II)

SECTION III

Plague

Cholera

Leprosy

Tuberculosis

SECTION IV

Malaria Control

Malaria General

Malaria Treatment

LIST OF ILLUSTRATIONS

CHAIRMEN AND RAPPORTEURS

SECTION III

Plague

onday, 5th December, 11 A.M to 1 P M
Chairman—Col J D Graham, C I E, I M S (B India)
Rapporteur—Lieut Col J Taylor, D S O, I M S (Burma)

onday, 5th December, 2 P M to 4 P M
Chairman—Lieut Col F P Mackie, O B E I M S (Bombay)
Rapporteur—Lieut Col J Taylor, D S O, I M S (Burma)

uesday, 6th December, 10 A M to 1 P M
Chairman—Lieut Col F P Mackie, O B E, I M S (Bombay)
Rapporteur—Lieut Col J Taylor, D S O I M S (Burma)

Cholera

uesday, 6th December, 10 A M to 1 P M
Chairman—Dr A R Wellington (Federated Malaya States)
Rapporteur—Lieut Col A J H Russell C B E, I M S (Madras)

uesday 6th December, 2 P M to 4 P M
Chairman—Dr A R Wellington (Federated Malaya States)
Rapporteur—Lieut Col A J H Russell C B E, I M S (Madras)

Dysentery Sprue and Intestinal Infections

ednesday, 7th December, 10 A M to 1 P M
Chairman—Prof K Shiga (Japan)
Rapporteur—Dr Digby Roberts (Assam)

ednesday 7th December, 2 P M to 4 P M
Chairman—Lieut Col J Morison I M S (Burma)
Rapporteur—Lieut Col J Morison, I M S (Burma)

Bacteriophage

ednesday, 7th December, 2 P M to 4 P M
Chairman—Lieut Col J Morison I M S (Burma)
Rapporteur—Dr Digby Roberts (Assam)

Leprosy

Thursday, 8th December 10 A M to 1 P M	*Chairman* —Dr J F E Bridger (Ceylon) *Rapporteur* —Dr E. Muir (Bengal)
Thursday, 8th December, 2 P M to 4 P M	*Chairman* —Dr Otto Schobl (Philippine Islands) *Rapporteur* —Dr F Muir (Bengal)

Tuberculosis

Friday, 9th December 10 A.M to 1 P M	*Chairman* —Major A Parker Hitchens (Philippine Islands) *Papporteur* —Dr A C Ukil (Bengal)

Bacteriology

Friday 9th December 10 A M to 1 P M	*Chairman* —Dr F d Herelle (Egypt) *Rapporteur* —Captain K R K Iyengar I M S (B India)
Friday 9th December 2 P M to 4 P M	*Chairman* — Dr A H Baldwin (Australia) *Papporteur* —Captain K R K Iyengar I M S (B India)

SECTION IV

Typhus like Diseases Leptospiræ etc

Friday 9th December 2 P M to 4 P M	*Chairman* —Dr Naosuke Onodera (Japan) *Rapporteur* —Lieut Col R Knowles I M S (Bengal)

Protozoology

Friday, 9th December, 10 A M to 1 P M	*Chairman* —Col S L Brug (Netherland Indies) *Rapporteur* — Lieut Col R Knowles I M S (Bengal)

Malaria Control

Monday 5th December 11 A M to 1 P M	*Chairman* —Sir Walter Fletcher K B F (Gt Britain) *Rapporteur* —Lieut Col C A Gill, I M S (Punjab)
Monday 5th December, 2 P M to 4 P M	*Chairman* —Sir Walter Fletcher K B F (Gt Britain) *Rapporteur* — Lieut Col C A Gill I M S (Punjab)

Malaria General

Tuesday, 6th December 10 A M to 1 P M	*Chairman*—Sir Malcolm Watson (Federated Malaya States) *Rapporteur*—Bt Col S R Christophers, C I E, O B E, F R S, K H P, I M S (B India)
Tuesday, 6th December, 2 P M to 4 P M	*Chairman*—Sir Malcolm Watson (Federated Malaya States) *Rapporteur*—Bt Col S R Christophers, C I E O B E, F R S, K H P, I M S (B India)

Malaria Treatment

Wednesday, 7th December, 10 A M to 1 P M	*Chairman*—Prof J W W Stephens (Gt Britain) *Rapporteur*—Major J A Sinton V C, O B E I M S (B India)
Wednesday, 7th December, 2 P M to 4 P M	*Chairman*—Prof J W W Stephens (Gt Britain) *Rapporteur*—Major J A Sinton, V C, O B E I M S (B India)

SECTION III.

PLAGUE.

CHAIRMAN'S OPENING REMARKS.

MONDAY DEC 5TH 11 A M 1 P M.

THE CHAIRMAN (COL J D GRAHAM, C I E, I M S, Public Health Commissioner with the Government of India) after welcoming the delegates to the home of plague and the country of the Plague Research Commission, of whom two of the original members Lieut Cols Gloster and Taylor, were present, said that certain explanations were necessary in regard to the nature of the session

In January last the Advisory Council of the Far Eastern Bureau of the League of Nations at Singapore appointed an Expert Plague Committee or Commission for international plague research and relegated the first discussion to the period when this Congress met in India

He accordingly invited the opinions of the League of Nations in Geneva, and the Secretary General of the F E A T M (Dr Deggeller) and as both were agreed on the advisibility of a joint meeting this meeting was agreed upon and was now about to take place

The work of the joint session would be done under two heads (a) the first would be the F E A T M session when all papers would be read and any discussions thereon would ensue, (b) the session would then go into sub committee as representing the League of Nations Expert Plague Commission The original members, as appointed at Singapore in January last would be added to by Dr Gautier arranging for representation of the nations not represented, and all who were likely to assist would also be co-opted The meeting would be held under the presidency of Dr Madsen

THE PRESENT POSITION OF THE PLAGUE PROBLEM

BY

LIEUT COL F P MACKIE OBE, IMS,
Director Haffkine Institute, Parel Bombay

WHEN I was honoured by the invitation to introduce the discussion on plague I had to decide whether I should review the subject as a world wide disease or whether I should select some particular aspect of the problem as it appears to us in India and study that aspect in closer detail

In view of the fact that we are privileged to entertain plague experts from other countries and to get the benefit of their views and of their advice, I decided to treat the subject from the wider viewpoint so that the discussion may range over a larger field and the experiences of the various workers in different countries may be presented to the Conference Owing to the limitations of time my remarks will have to be superficial and incomplete, but I hope they may serve their purpose which is to stimulate discussion on plague in all its aspects

I THE RODENT FACTOR

Plague is primarily a disease of rodents and the involvement of man is incidental almost accidental The countries where plague is unfamiliar or in sparsely inhabited countries the disease amongst rodents often passes unnoticed till human plague occurs and calls attention to the mortality amongst rats

The transmission of infection from man to man and from man back to the rat must be exceedingly rare though it is possible that carnivorous rodents by feeding on a plague cadaver may occasionally contract the disease

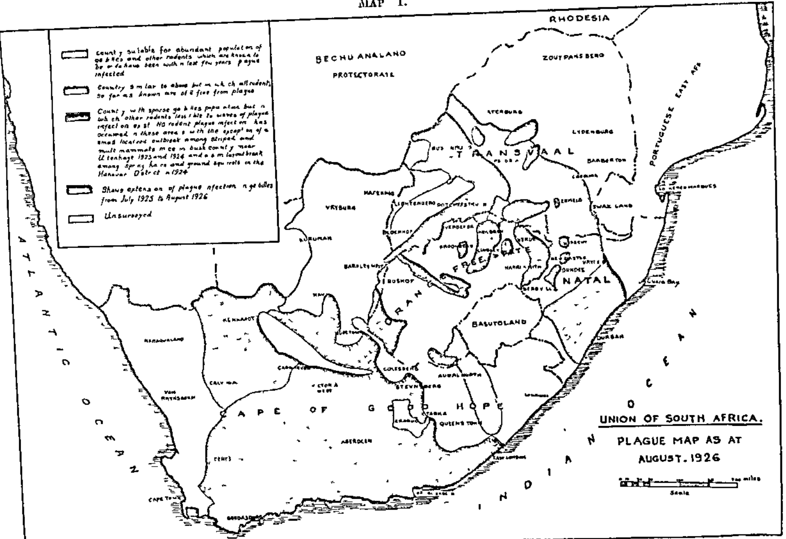
Following on the successful elucidation of the problem in India it was supposed that the disease was always carried by domestic rats especially by *R norvegicus* and *rattus*, but more recently it has been shown that in Manchuria and Transbaikalia the rodent responsible is the tarabagan in South Russia spermophile rodents such as the susliks in South Africa gerbilles and other veldt rodents and ground squirrels in California

This extension of the plague menace requires more detailed notice

Conditions in India —The Plague Commission working in Bombay in 1905 *et seq* found that *R norvegicus* was nearly thrice as liable to infection as *R rattus* and in one year's examination nearly 18 000 (*R rattus* 4 381, *R norvegicus* 13 377) out of 117 000 rats were plague infected

It was found that during the off season acute plague persisted amongst the rats but to a much less extent than during the plague season This seasonal recrudescence is an interesting and important fact and may be due in part to the presence of a young and non immune rat population The epizootic in Bombay starts amongst the *norvegicus* and is followed after an interval of about a fortnight by a rise of plague amongst the *rattus* population

MAP I.



UNION OF SOUTH AFRICA.
PLAGUE MAP AS AT
AUGUST. 1926
Scale
Unsurveyed
RHODESIA
BECHUANALAND
PROTECTORATE
ZOUTPANSBERG
PORTUGUESE EAST AFR
LYDENBURG
BARBERTON
TRANSVAAL
SWAZI LAND
VRYBURG
MAFEKING
KURUMAN
BLOEMHOF
BOSHOF
FREE STATE
ORAN
BASUTOLAND
NATAL
DURBAN
HARRISMITH
DUNDEE
VRYHEID
KENHARDT
NAMAQUALAND
CALVINIA
VAN RHYNSDORP
CAPE TOWN
ABERDEEN
COLESBERG
ALIWAL NORTH
QUEENS TOWN
CAPE OF GOOD HOPE
ATLANTIC OCEAN
INDIAN OCEAN

The greater incidence amongst *norvegicus* is apparently due to a greater degree of flea infestation but despite this fact the Commissioners conclude that in Bombay it is the *rattus* epizootic which determines the human epidemic

In the interior of India particularly in the villages the prevalent and often the only species of rat is *rattus* In the off season rats are found which have resolving plague lesions mostly in the form of abscesses from which living *B pestis* can be recovered and it is probable that such rats play a part in carrying over plague from one season to another

What part does rat immunity play in the matter? Dr Naidu gives figures to prove the high immunity of Bombay rats to plague (a feature noted previously by the Plague Commission) with the result that for many years the use of Bombay rats for experimental purposes at the Haffkine Institute has been discarded and susceptible animals imported from Madras At first sight it appears paradoxical that the rat population is most susceptible where there is no plague and this has been observed amongst ground squirrels and other wild rodents in other parts of the world The explanation must be that the conditions of a persistent or recurrent epizootic induces partial immunity which is hereditarily transmitted to the surviving animals

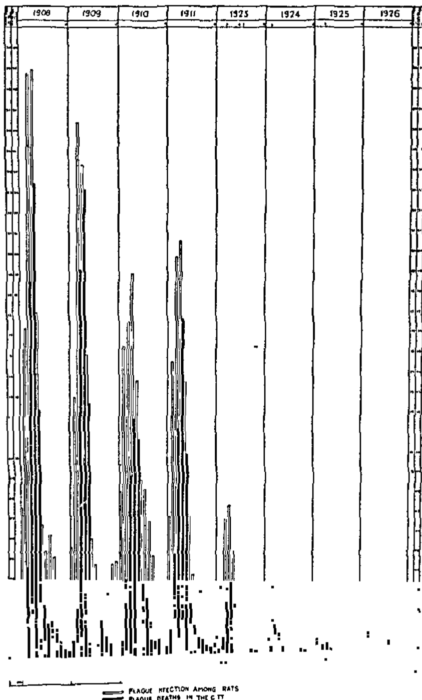
Chart I shows the percentage of infected rats found on examination at the Haffkine Institute and also the ratio of infected rats to human cases

The conditions in *South Africa* are interesting and distinctly disturbing The infection was apparently originally ship borne and appeared amongst the shore rats in or near the ports and human infection occurred in the usual way Of recent years it has not spread appreciably in the ports or amongst the domestic rat population but has lit up in the interior of the country where domestic rats are rare or unknown For information on these matters we are indebted to the recent monographs by Mitchell Harvey Pirie Ingram and Murray They have shown that a number of wild rodents which abound on the veldt particularly some species of gerbilles and also multimammate mice Karroo rats squirrels and hares are all highly susceptible to plague and are playing an active part in the spread of the disease

The common gerbille is said to be the most active carrier whilst the Namaqua gerbille an allied species is relatively unsusceptible and may act as a barrier to plague diffusion Map I is taken from the South African report and shows the distribution of these mammals and will impress upon us the magnitude and the importance of the aspect of the problem It raises the question as to whether we in India have paid sufficient attention to the possibility of rodents such as squirrels and hares playing a part in the spread or maintenance of plague These wild rodents in South Africa are capable of carrying on an epizootic and of involving man in the entire absence of domestic rats

It is not clear how the infection is carried over from one season to another for Mitchell and Pirie have not succeeded in demonstrating chronic or residual plague amongst these rodents

CHART I.



Percentage of infected rats found at the Haffkine Institute and the ratio of rat to human cases in Bombay.

In view of the scarcity of such lesions even in a saturated plague area like Bombay the probability of this mode of transmission cannot be excluded in the South African problem

The possibility of the flea itself being the carrying over agent must not be lost sight of Elton's hypothesis of the recurrent epizootics which occur amongst rodents and which serve to keep their populations in reasonable proportion to the available food supply may have an important bearing on the problems of plague here and elsewhere With this hypothesis in view Mitchell and Pirie go so far as to forecast the possibility of a recrudescence of plague in South Africa during the present or the coming year Writing of the most recent epidemic in the north west of the Cape Province it was stated to possess unusual epidemiological features in that —

(a) there was a difference in the species of responsible rodents
(b) the epidemic broke out unexpectedly in a fresh part of the country and
(c) the epidemic appeared in the winter whereas previously they had started in the summer

Facts like these show that we have much to learn concerning the fundamentals of plague epidemiology and it is evident that the problem in Africa and other places where the population is scattered and domestic rats absent presents quite different features to those with which a quarter of a century of work has familiarized us in India

Turning to the question of plague in *California* a very similar problem presents itself The disease appeared first in the Port of San Francisco which was infected by ship rats from some Asiatic port and the earlier cases were associated with these rodents Later however, cases of plague occurred where rats and mice were absent and it was found that an epizootic was present amongst ground squirrels in the interior at a distance from the port and the coast line These squirrels were found to be highly susceptible to plague which could be transmitted to them through the medium of rat fleas (*C fasciatus*) and that in nature the two classes of rodents mixed at or near the coast line

McCoy examined 216 naturally infected squirrels and found that 75 per cent had a bubonic infection (mostly cervical) and what is more important nearly 14 per cent suffered from chronic or residual plague He also observed as we have in Bombay that squirrels found in an infected locality show a high degree of resistance to artificial infection compared with the much higher susceptibility of those caught in plague free areas

It appears that apart from this ground squirrels are less susceptible to plague than rats and the proportion of chronic or residual plague amongst them is higher suggesting that they form a smouldering kind of union between epizootics separated by space or time McCoy also believes that human plague is less severe when contracted from a squirrel than from a rat

The course of events in California has been very similar to what has occurred in South Africa and is what may be expected to happen in other places where similar conditions exist —

(1) The importation of infected rats or their fleas by sea from a previously infected port

(2) An epizoötic amongst port rats and the occurrence of human cases from this source

(3) The disappearance of infected rats (or at any rate of human plague cases) from the port due to active preventive measures aided perhaps by such natural factors as the meteorological conditions which determine flea infestation

(4) *A lull in the rat and human cases whilst infection is filtering through to the indigenous rodents*

(5) The establishment of epizoötic or enzoötic plague amongst the indigenous rodents without assistance from or in the absence of the domestic rat population

(6) *Cases of human plague amongst those of the population brought* closely into contact with wild rodents (e g , gerbilles and ground squirrels) and in localities distant from previously known foci of infection

In *Northern Asia* the problem is again similar though the original rat infection, if it ever existed, cannot now be traced The origin of plague in this area is unknown but it is considered by Wu Lien Teh and others to be the primitive focus of *B pestis* infection whence spread the present pandemics some thirty years ago The rodent responsible is the tarabagan (*Arctomys bobac*) and possibly other smaller rodents of the steppes

The exhaustive study of pneumonic plague by Dr Wu Lien Teh, recently published by the League of Nations is a mine of information on this and other aspects of the plague problem and I have made free use of this comprehensive monograph However much the clinical type of human plague found here differs from that seen in warmer climates, the march of the epizootic is similar to that already noted elsewhere The habits of tarabagans provide facilities for transmission from one to the other by their ectoparasites and Wu Lien Teh has no doubt that this is the method almost invariably responsible The post mortem signs of plague are very similar to those in rats and so is the distribution of buboes which are mainly cervical The habits of the animals being as they are, it is not surprising that chronic or residual plague lesions are very frequent

It is probable that the hibernating habits of the animal during which time the body temperature falls and the vital processes are reduced to a low ebb, facilitates or predisposes to the perpetuation of infection in a chronic or smouldering type Caseating lesions containing *B pestis* foci of the organism in large masses in the liver and lungs are some of the remarkable conditions described as occurring in cases of residual plague in hibernating animals

Such conditions may well explain the recurrence of the epizootic at the end of the long hibernation On the other hand it has been suggested that carrying over is effected by some of the smaller rodents which act as intermediaries between colonies or areas of tarabagan distribution The first persons to be infected with plague are the tarabagan hunters or those who skin or handle their carcases and there is no reason to doubt that the transmitting agent is the tarabagan fleas which readily bite man and the initial human infections are bubonic and not pneumonic

In *South Russia* where plague occurs the sequence of events is precisely similar Here the responsible rodent is a small grain eating animal the suslik, and also certain domestic and field mice which live on the steppes It has been noted that the small susliks though highly susceptible to *B pestis* infection by the subcutaneous method are also easily infected by feeding on plague material In such cases they develop an intestinal type of plague characterized by bloody diarrhœa in which *B pestis* is present and which may develop into the bubonic, septicæmic or pneumonic form The disease is spread amongst these animals both by the agency of ectoparasites and also by feeding on the bodies of their dead comrades Chronic or residual plague is common amongst these animals and experiments have shown that in hibernating animals infected with plague the disease may run a course of five months whereas non hibernating animals die in two to seven days This together with their cannibalistic habits would explain how the epizoötic may be carried over the winter season and reappear when the animal emerges in the spring

Dr Wu Lien Teh in the monograph referred to has compiled a list of no less than 38 rodents other than domestic rats and mice which have been proved to be susceptible to plague and the majority of which have been found in some part of the world to be naturally infected In such a way has the plague problem been widened since the days when we believed the domestic rats were essential to the occurrence and spread of human epidemics

II The Insect Carrier

We know that under ordinary conditions bubonic plague is carried by species of rat fleas and it seems equally clear that amongst other rodents in Manchuria South Africa California and South Russia the fleas associated with these wild rodents are also mainly responsible for transmission Compared with the large amount of work which has been done on rats and rat fleas there is still much to be done in working out the exact association of these wild rodents and their ectoparasites The problem is similar to that which exists in the case of malaria and various species of Anopheles It has taken years to determine which are the most effective carriers of malaria and to distinguish them from others which may not have the carrying power to start an epidemic, but are able to contribute to its persistence and to learn that there are other species which for no known reason are unable to carry malaria in nature or even under laboratory conditions Since the days of the Indian Plague

Commission we have known that plague was transmitted by rat fleas without much discrimination of species, but thanks to the work of Rothchild, Hirst, Cragg and many others we now know that the common rat fleas of India are not of one species and that they vary considerably in carrying power Similar differences doubtless exist amongst wild rodents and taking the example of susceptible and unsusceptible races of gerbilles in South Africa, their variations in this respect may be a function of their own natural immunity or it may be due to the species of ectoparasite they carry Although flea transmission is the vastly predominating agency from rat to rat and rat to man, we must not lose sight of the fact that alimentary transmission is possible under certain conditions and plague may be transmitted from rodent to man by skinning or handling infected corpses without being of necessity through the medium of fleas In this connection Hirst says —

'We must guard against the unwarranted assumption that ectoparasites such as fleas play an essential part in the spread of plague amongst every variety of rodent under all conditions'

Reference has already been made to the spread of plague amongst susliks in South Russia in the form of an intestinal infection characterized by bloody diarrhœa, and, speaking of South Africa, Mitchell and Pirie say that in the case of the gerbille there is evidence that spread of plague by feeding may play a considerable rôle

The question also arises whether the type of human plague differs according to the source or of the vector The observation of McCoy has already been cited that the type of human plague when derived from the ground squirrel through its fleas was less severe than those infected from the rat *cheopis* source Hirst says 'It is noteworthy that these extensive enzootics amongst miscellaneous mammals with miscellaneous species of fleas have all occurred in relatively cold climates and they have all been associated with outbreaks of pneumonic plague in man' Given that chronic or residual plague in rodents is responsible for carrying over from one season to the next, how is the epidemic started? Flea transmission postulates a condition of septicæmia in the rodent and this certainly does not ordinarily exist in these chronic cases Two explanations are possible—one that a certain number of chronic plague rats flare up and give rise to septicæmia and so infect the fleas or *that the chronic plague rat* dies in that condition and is eaten by his comrades which develop septicæmic plague and so allow the flea to come into action

The question as to whether other ectoparasites of rodents can convey plague, Wu Lien Teh quotes an instance where a healthy tarabagan was infected from a diseased one by the transfer to it of 40 lice

Hirst suggests that blood sucking *acari* may play a part and, in the case of human plague, bugs and lice have been suggested as carriers Another point of interest is, what effect has the plague bacillus on the insect carrier itself? We all know the work of Bacot and Martin on the '*blocking*' of fleas by pullulation of *B pestis* in their upper alimentary tract and the importance of this phenomenon in the transmissive power of the affected insect Other experiments

have been made which show that the cœlomic fluid of bugs and lice becomes plague infected and that these insects die as one might say of septicæmic plague It must not be forgotten too that fleas hibernate as well as their hosts and may retain their infection under suitably reduced temperature for as long as 208 days

Turning now to the problem of plague transmission amongst domestic rats (*R norvegicus* and *R rattus*) we find ourselves on more familiar ground The work of the Indian Plague Commission laid sure foundations to which many valuable additions have been made in Ceylon India and elsewhere Particularly I would refer to the admirable monograph by Fabian Hirst of Colombo recently published on the parasitology of plague which summarizes most of our knowledge of this side of the problem As far as it concerns India the important question is as to the relative efficiency of the three principal rat fleas as transmitters of plague and whether the distribution of the three species *Xenopsylla cheopis*, *astia*, and *brazilensis* explains the remarkable irregularity of plague incidence in different parts of India Maps handed round show the general distribution of plague in India and the relative frequency of *cheopis astia* and *brazilensis* as far as we know it

Hirst holds that the distribution is mainly dependent on the relative frequency of the first two species of fleas, the former *cheopis* being a good plague carrier and *astia* a relatively ineffective one Experiments by workers in India agree that *cheopis* is certainly a better carrier than *astia* but that the latter can by no means be ignored as a carrier and indeed that under certain climatic conditions it may be very effective Hirst never observed the phenomenon of ' blocking ' in *astia* but frequently in *cheopis* and concluded that the greater efficiency of *X cheopis* as a vector may be attributed to its greater viability and its more effective biting power He adds that rat fleas of different species may be equally efficient vectors of rat plague their relative efficiency varying according to the climatic conditions

' Speaking roughly a *cheopis* index of 1 per rat seems compatible with the continuous spread of epizoötic plague at a temperature of about 75°F and a saturation deficiency of less than 0 3 inch '

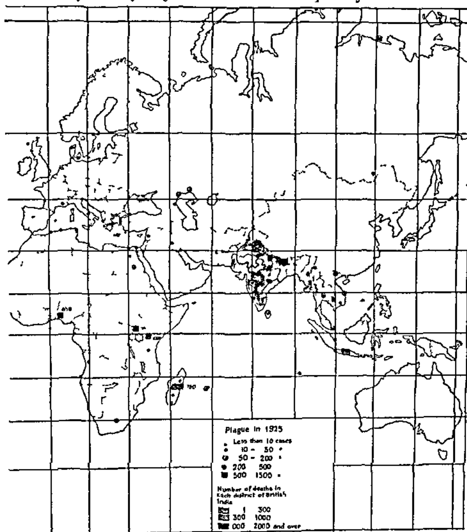
As regards the vexed question of the relative importance of flea species to climatic variation Hirst is of opinion that ' Climatic conditions limit but do not govern the geographical distribution of rat fleas and in his general conclusion on the subject he adds— X *cheopis* is the prime vector of bubonic plague between the domesticated species of rats and between these rats and man Other species of *Xenopsylla* and various species of *Ceratophyllus* may temporarily extend an epizootic initiated by X *cheopis* in *cheopis* free territory or prolong its seasonal period when *cheopis* is absent

Liston (Milroy Lectures) writing of the climatic factors concludes that the most favourable temperature for transporting infected rat fleas is about 50°F when the atmosphere is damp and the most unfavourable temperature for this purpose is one above 80°F or even at a lower temperature when the atmosphere is dry Suitable temperatures promote the activity of fleas and their multiplication and prolong their life, so that they are more numerous, more active in feeding and

living longer and can be transported more readily to distant places in a living condition That meteorological conditions play a very important part in India there can be no doubt and charts have been prepared showing the close correlation between the rise and fall of epidemics and the conditions of temperature and humidity The species of fleas under discussion are markedly and differently affected by various

MAP II

Distribution of Cases of Plague in the Eastern Hemisphere of the World in 1925



The world distribution of plague (Annual Report of the League of Nations)

climatic conditions so that the two phenomena are interlocked and all attempts to explain the distribution of plague in India will be futile unless due regard is given to the two prime factors, viz , the climatic condition and the relative preponderance of flea species

III The Human Factor.

Little time remains to discuss this import int aspect of the problem and restrict myself to handing round a few charts and maps which speak for ther

Map II from the 1925 Annual Report of the League of Nations sho world distribution of plague and its principal endemic area in and around India

Chart II

Course of Plague 1922—1925

SCALE ▭ = 1 000 cases

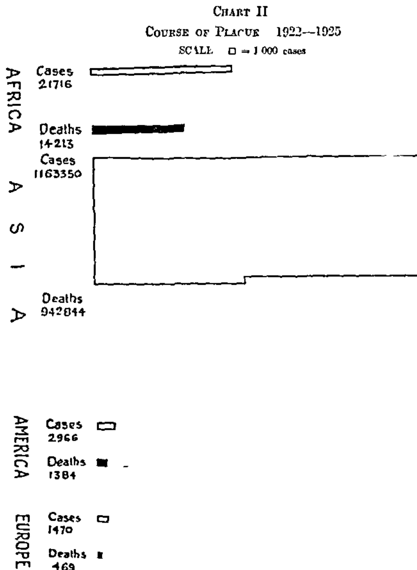
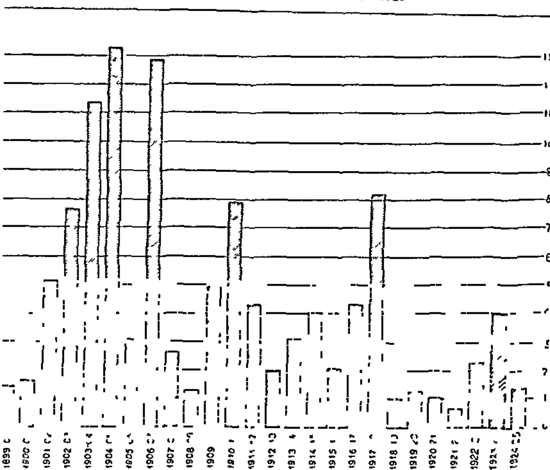


Chart V shows the plague mortality which has occurred *in India during* the last 27 years

CHART V

PLAGUE MORTALITY IN INDIA 1898—1925



The plague mortality in India during the last 27 years

Map III shows the distribution of plague in India during 1925 (copies from the Annual Report of the League of Nations)

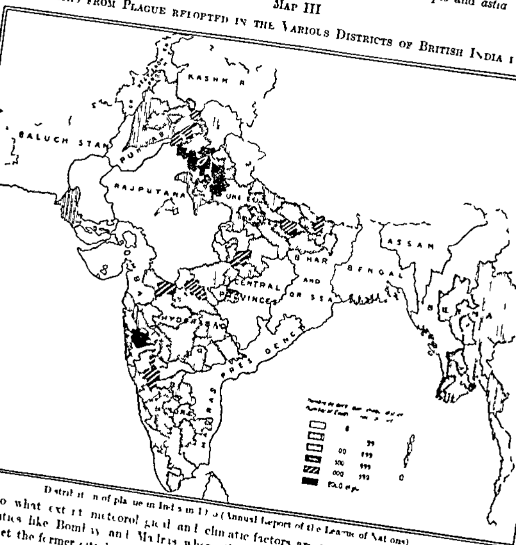
The most striking point about this distribution map is not so much the con*sideration of the infected areas but of the large tract of country which has remained* free from infection

From a study of Chart V we might flatter ourselves that we were over the *worst of the epidemic for in recent years the number of cases has been declining,* whilst according to some figures the case mortality is also getting lower But when we regard Map III we find that the disease is not declining for want of com*bustible material and we are made to realize how deficient is our knowledge of the*

natural history of the disease in that we have no certain explanation as to
large areas in that map are white whilst those nearby are shaded This is or
the problems we have to solve and India is the place to solve it Is it due to s
variation in the rat population or to the distribution of X cheopis and astia

Deaths from Plague reported in the Various Districts of British India i

Map III



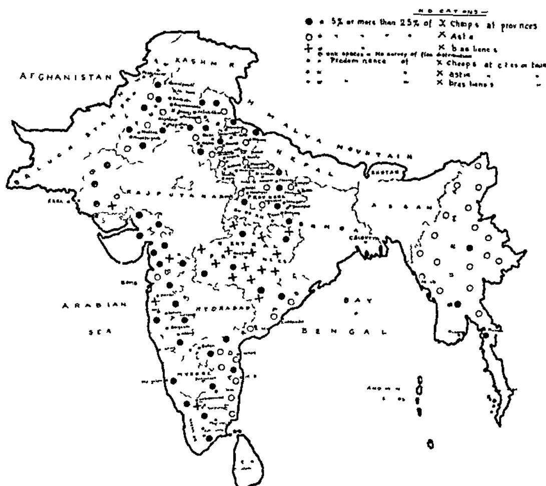
Distribution of plague in India in 1923 (Annual Report of the League of Nations)

to what extent meteorological and climatic factors are responsible? Take two
cities like Bombay and Madras where the climatic conditions are very similar
yet the former city has been ravaged by plague year by year and Madras has been
spared despite frequent importations The fact that the cheopis index is high in
Bombay whereas Madras shows a 100 per cent astia index would seem to b

deciding factor and as Hirst says ' The discovery of a pure *astia* population on the rats of that remarkably plague immune region (Madras) remains the most significant fact in the parasitology of plague in India '

The flea distribution for India is as yet incompletely worked out but Map IV taken from Hirst's memoir and other sources is handed round and may be compared with the plague distribution in Map III

MAP IV.

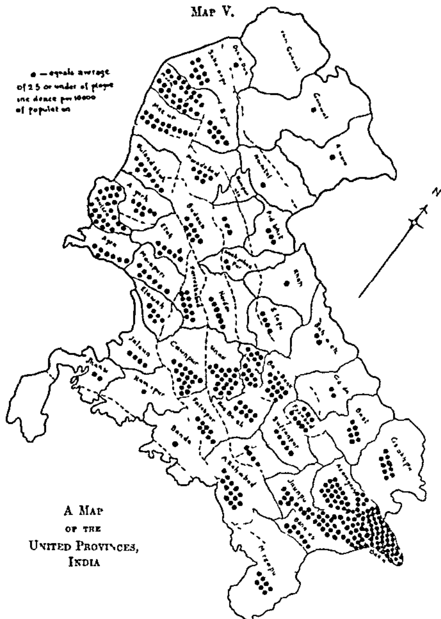


Flea distribution in India (after Hirst and others)

Map showing percentages of *Xenopsylla cheopis* X *astia*, X *brasiliensis* found on rats (irrespective of their species) in five out of the provinces of India from each of which at least 300 fleas were examined It also shows the predom ... h at least 100 fleas were examined ... refer to interrupted periods from ... om April 1925 to August 1926, etc

Map V has been prepared by Dr Chitre to show the distribution of plague and of the principal species of fleas in the various districts of the United Provinces from Dr Mithal's records.

MAP V.

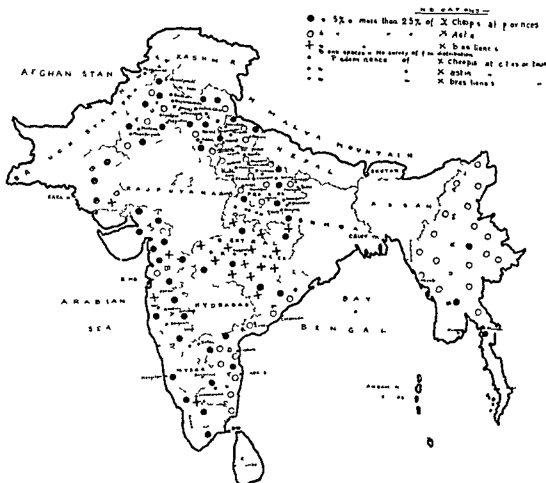


Showing average plague incidence for each district per 10 000 of population for the period from 1903 to 1925

deciding factor and as Hirst says ' The discovery of a pure *astia* population on the rats of that remarkably plague immune region (Madras) remains the most significant fact in the parasitology of plague in India '

The flea distribution for India is as yet incompletely worked out but Map IV taken from Hirst's memoir and other sources is handed round and may be compared with the plague distribution in Map III

MAP IV.

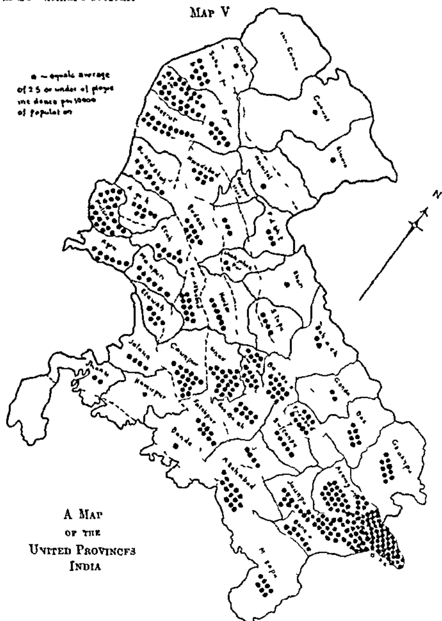


Flea distribution in India (after Hirst and others)

Map showing percentages of *Xenopsylla cheopis* X *astia* X *brasiliensis* found on rats (irrespective of their species) in five out of the provinces of India from each of which at least 300 fleas were examined It also shows the predominating out of three species at certain towns and cities from which at least 100 fleas were examined The data are collected from reports of Cragg Hirst Mithal, etc and refer to interrupted periods from December 1919 to October 1920 from March 1921 to July 1921 and from April 1925 to August 1926 etc

Map V has been prep^red by Dr Chitre to show the distribution of plague and of the principal species of fl\as in the various districts of the United Provinces from Dr Mithal's records.

MAP V



Showing average plague incidence for each district per 10 000 of population for the period from 1903 to 19'5

MAP VI

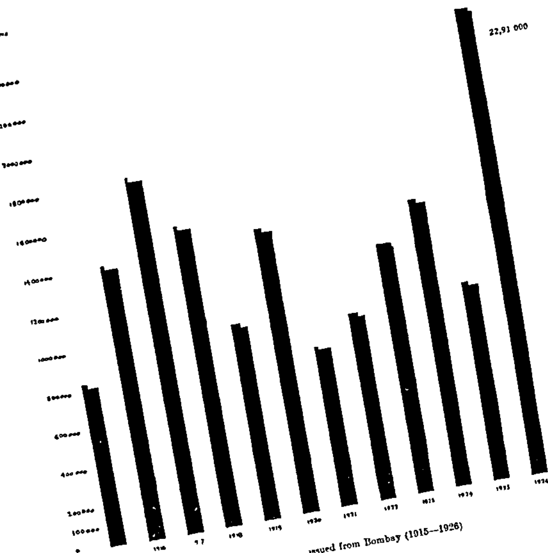


Showing percentage of *Xenopsylla cheopis* fleas amongst those collected by Dr Chitre ar Dr Mithal from certain selected places of the districts to which they refer The fleas we collected during the period from April 1925 to end of August 1926 The percentage *Xenopsylla cheopis* for respective districts only are shown

F. P. Mackie.

I have no time to deal with the subject of plague prophylaxis nor with the measures directed towards the extermination of rodents and their parasites. During the thirty years during which Haffkine's vaccine has been used in India, nearly a million doses a year have been used and a large amount of evidence has

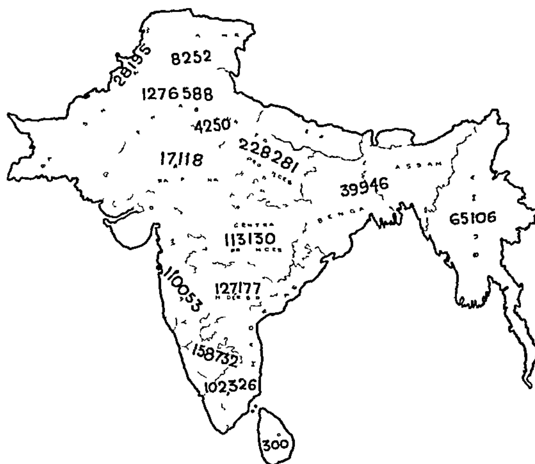
CHART VI



...issued from Bombay (1915—1926)

been collected on the prophylactic value of this measure. Chart VII has been compiled from statistics collected over a number of years from observations in which it was possible to note carefully the incidence of the disease amongst inoculated and non-inoculated persons. They should convince any impartial observer of the undoubted value of Haffkine's prophylactic.

MAP VII



Distribution of Haffkine's prophylactic to provinces in India during 19[illegible]6
Total number of doses—[illegible] 415 38[illegible]

Further evidence on this aspect and on the effect of sera, bacteriophage and of chemotherapy will be dealt with by Dr. Naidu in a separate paper.

In the discussion which will follow this paper I hope that the views of experts from other countries will throw light on the interesting and important problems which still await solution.

CHART VII.

THE PROPHYLACTIC VALUE OF HAFFKINE'S VACCINE AS REVEALED BY STATISTICS

TABLE I.

Figures are taken from the Reports of the Bombay Bacteriological Laboratory for the years 1902—1916

	Population	Attacks	Per cent	Deaths	Per cent	Survivors to attacks per cent.
Uninoculated	2,629,072	56,240	2.13	48,080	1.8287	14.51
Inoculated	1,739,193	3,164	0.18	1,209	0.0695	61.79

TABLE II

From the Statistics of Inoculation in the Town of Ilkal in Bijapur District where a careful house to house inquiry was made. Report of the Bombay Bacteriological Laboratory for the years 1915 and 1916

	Population	Attacks	Per cent	Deaths	Per cent	Survivors to attacks per cent
Uninoculated	39	20	51.2	19	48.71	5.0
Inoculated	63	4	6.34	1	1.587	75.0

TABLE III

Immunizing Value of the Haffkine's Plague Prophylactic in Rats

Years	Number of experiments	Number of brains used	Number of rats used	Total deaths in 7 days following on inoculation	Percentage mortality	Survivors	Total deaths in 15 days following on infection	Survivors percentage immunity
1923—25	49	29	1,015	237	23.3	778	515	33.0
				Controls		482	450	6.7
1926	82	67	3,350	455	13.5	2,895	2,030	29.8
				Controls		360	336	6.6
1927 To the end of October	46	40	1,710	190	11.1	1,520	968	36.3
				Controls		210	198	5.7
Total results	177	136	6,075	842	11.5	5,193	3,513	32.3
				Controls		1,052	984	6.4

PROBLEMS OF PNEUMONIC PLAGUE

BY

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TABLE OF CONTENTS

A INTRODUCTION

IT is gratifying to note that the problems of pneumonic plague have received within recent years the attention they deserve Nevertheless, considerable divergencies of opinion still exist on some important points A discussion of these questions at such a meeting as this where plague experts from different countries are assembled is therefore welcome The present occasion is particularly auspicious, since for the first time we are met in the country where—in addition to immortal research work done upon bubonic plague—the fundaments were early laid for our modern conception of the pneumonic form

B HISTORICAL SKETCH

Before following the trend of events from this epochal period onwards, it is necessary to trace the history of pneumonic plague in former times

(1) *Before the fourteenth century*—Although the pneumonic form had occurred side by side with bubonic plague since time immemorial, but little evidence is now available regarding our early knowledge of the former No doubt occasional cases of lung pest were confounded with the prevalent bubonic ones, while outbreaks with mainly pneumonic manifestations might not have been taken for plague at all It is not surprising therefore that all we can establish is that the pneumonic type was in all probability met with during the pandemic known as Justinian s plague (sixth century), especially at

Constantinople, and that an undoubted outbreak of this kind was rampant in Provence, A D 1329

(2) *The Black Death* (1346—52) —In spite of the scepticism still professed by some modern investigators there is no doubt that the pneumonic type played an important rôle in this worst of all known pandemics. It would lead us too far to discuss the evidence available in this respect from different countries. Suffice it to summarize that several contemporary recorders differentiated clearly between bubonic and pneumonic plague as manifestations of one and the same disease, and also noticed that those with lung affections were more infective and died quicker than the others One prominent fact is that the pneumonic type was most prevalent at the beginning of many local outbreaks, but in only a few localities did the disease continue to rage in this form As a rule it assumed a bubonic character after a few months This change occurred often, though by no means always, with the approach of warm weather

(3) *From the end of the fourteenth to the beginning of the nineteenth century* —One would expect that the clear differentiations between the various forms of plague, arrived at during the great pandemic known as the Black Death, had become a matter of common knowledge Here, however as in other branches of medicine, we see that the truth, recognized only by a few advanced thinkers, soon became forgotten again In this case it seems to be due mainly to the fact that—as long as plague was frequently rampant at the time soon after the Black Death—contemporary observers ceased to dwell upon an evil which had become familiar to everybody Later on descriptions were again voluminous, but confined almost entirely to the bubonic variety, pneumonic features, if present, having apparently been disregarded This was the easier, because pneumonic manifestations, though undoubtedly met with in this period,* seem to have been quite rare It may be added that the proofs we possess in this regard are almost entirely restricted to Europe I know of only two testimonials pointing to their existence in the East

(a) Delbreil states, without giving any authority, that the pneumonic type was met with in India during the sixteenth century

(b) *The Ku Chin T u Shu* (An encyclopædia published in Peking in 1726) says that in the year 1644 a great pestilence raged at Lu an in the south east of Shansi Province 'Those attacked had hard lumps grow on the neck or arm, like clotted blood Whole families perished In some cases the victims vomited blood suddenly and expired'

(4) *In the first half of the nineteenth century* —This unfamiliarity with the pneumonic type of plague, which continued to be rare, reached such a degree that it was even doubted if the Black Death, where lung symptoms were frequent, was plague at all It reflects great merit upon authors like Hecker, Webb and

* For fuller information on this and other problems mentioned in this article, see my 'Treatise on Pneumonic Plague', Geneva, 1926 and 1927

Hirsch to have proved the plague nature of this pandemic in general and of its pneumonic manifestations in particular Webb *and Hirsch at the same time* demonstrated the occurrence of the latter form in two modern Indian outbreaks namely 1812—21 and 1836—38, here the pneumonic type was excellently described by observers like Gilder and Whyte Forbes Keir MacLean, etc However this form was considered peculiar to the Indo Chinese strain' of plague and absent in Western Asiatic' or Levantine' plague

(5) *In the second half of the nineteenth century*—The pneumonic type was met with occasionally in particular the well known outbreak at Vetlianka (south east Russia) of 1878 79, but only a few keen and open minded observers like Muench were convinced of the plague nature of the lung cases seen during this epidemic It is to the everlasting credit of Childe (Bombay) that he first established definitely at the beginning of the present pandemic the pneumonic form as a special entity of plague At this time pneumonic features were quite often met with

(6) *From* 1896—1911 —On the whole lung pest was considered as an interesting but rare variety of the disease until the great Manchurian epidemic of 1910 11 showed that it might assume dimensions comparable to those of the Black Death Still the fact that this epidemic occurred during severe winter together with other data available at the time, suggested to many observers that pneumonic plague was a disease peculiar to cold climates only

(7) *From* 1911 *to our times*—It was only within recent years that this erroneous assumption has been revised It is agreed now that the disease may also occur —

(*a*) during hot seasons,

(*b*) in countries with a warm and even tropical climate

There is no doubt that pneumonic plague is frequently encountered and an authority like Jorge (Lisbon) considers it to be more rampant now than earlier in the present pandemic This greater frequency is perhaps more apparent than real far more attention being now paid to this type than was the case a few decades back

C Epidemiology of Pneumonic Plague

(1) *Rise of Epidemics*

(a) *How do pneumonic epidemics start?*—Some authors have not emancipated themselves from the idea that pneumonic outbreaks arise *de novo* i e, that the first victim already which became infected directly from the rodents or through coming in touch with contaminated objects may display features of primary pneumonic plague In the course of our researches I have paid special attention to this aspect I had the opportunity to analyse not only the ætiology of the outbreaks in Transbaikalia Mongolia and Manchuria but to consult practically all reports available from different parts of the world The result of this study is that only in exceptional instances such a rise *de novo* is likely Therefore the existence of outbreaks of this

kind cannot be totally denied But it must be emphatically stated that they are rare exceptions and not the rule As proved by the ample experience gathered at the principal foci of pneumonic plague like Egypt Madagascar South Russia Transbaikalia and Manchuria pneumonic plague usually originates from bubonic cases with secondary lung involvement Some authors are inclined to ascribe a similar role to cases with skin or septicaemic plague It is evident that even when the former (skin) cases could act in such a manner, they are too rare to be of any practical importance Those labelled as septicaemic plague are more frequent but their role is also not firmly established One point which should always be kept in mind is that one cannot be sure of the purely septicaemic character of a case even where no buboes have been *noted* *

(b) *Why do pneumonic epidemics arise?*—While it can thus be seen that the problem as to how pneumonic outbreaks arise is satisfactorily settled the question why such epidemics arise is still a justly much-debated one Different theories are advocated in this respect which may be classified as follows —

(α) Bubonic and pneumonic plague being due to one specific organism, are varieties of one and the same disease The rise of epidemics of the latter type is due to chance or solely extrinsic causes

(β) The rise of pneumonic epidemics is due to intrinsic causes

(γ) The question is still an open one certain possible causes for the rise of pneumonic epidemics being known but not sufficiently investigated to warrant hard and fast conclusions

Ad α —Allusion has been made already to the theory that pneumonic plague is prevalent only in cold climates cases which may develop under warm weather remaining sporadic and not leading to epidemics As said above such views have not been borne out by recent observations lung pest in epidemic form having been found under all sorts of climates and in diverse countries In general there is much evidence against the 'extrinsic' theory It is known for instance that the disease is frequent in certain countries only rare or absent in others with no milder climate or better social conditions Another important fact is that in some areas the pneumonic type was frequent for a period immediately after introduction of infection to become rare or absent later on Again no change in climatic or social conditions exists which might be responsible for this peculiarity

Ad β — One is tempted to assume that a fundamental difference exists between bubonic and pneumonic plague Different theories have been formulated in this respect Most of them are of historical interest only and need not detain us One point which must be emphasized is that it has not been possible so far to find any marked difference in the bacilli causing bubonic and pneumonic plague respectively, both kinds of strains are as far as can be determined by present day

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* This aspect has been recently emphasized by Girard of Madagascar (*Bull Soc Path Exot* 1927 p 632) and is the more interesting because formerly septicaemic cases were thought to be a frequent cause of the pneumonic outbreaks in Madagascar

methods identical It is as easy to cause pneumonic plague in suitable animals by inhaling them with a strain derived from a purely bubonic human case, as to produce bubonic plague in laboratory animals by infecting them cutaneously or subcutaneously with fresh pneumonic strains or even directly with material obtained at the post mortem of lung victims

There remains only one theory, once held in India and recently advocated by Norman White which must be discussed According to him, 'the plague bacillus *alone* does not and cannot, cause widespread epidemics of pneumonic plague and it seems more than probable that there is an additional organism at work—in other words, the plague bacillus in symbiosis with another organism is responsible for epidemic manifestations of pneumonic plague, which is a disease *sui generis*' Nicolle and Gobert argued that the filterable virus of influenza might act together with the *B pestis* in this respect and based their opinion upon epidemiological experience in Tunisia

In my belief such theories are untenable and cannot explain the rise of pneumonic epidemics for the following reasons —

(*i*) The pneumonic type in practically all outbreaks does not arise *de novo* but is traceable to bubonic plague, the connecting link being cases with secondary lung involvement

(*ii*) In most big pneumonic epidemics, a few bubonic cases are observed Often such patients received infection more or less directly from pneumonic plague patients (or from corpses at post mortem) and yet developed bubonic plague, sometimes in quite mild form

(*iii*) Instances are known where simultaneously existing cases of bubonic and pneumonic plague could be traced to one and the same source

(*iv*) Influenza is not always prevalent at the time of pneumonic epidemics In fact only very few instances are on record where simultaneous existence of both diseases was noted In many others where special attention was paid to a possible co existence of influenza during pneumonic epidemics the former disease was conspicuous by its absence

(*v*) No line can be drawn between sporadic and epidemic manifestations of pneumonic plague, as Norman White seems to do Whether pneumo pest *spreads* or not, depends upon extrinsic and not upon intrinsic factors

(*vi*) In general it should be kept in mind that the problem of mixed infection in plague is a very complicated one Therefore, one cannot be too cautious in accepting any theory of this kind even when it *seems* to be supported by epidemiological or laboratory data

Ad γ — The peculiar distribution of pneumonic plague both from a geographical standpoint and in relation to the time of occurrence in certain areas as discussed under (*a*) suggests that the difference between bubonic and pneumonic plague cannot be such a superficial one as advocated by the supporters of the

'extrinsic' theory To attempt a solution of this problem it may be well to analyse, step by step, the factors which might contribute to give an outbreak its pneumonic character If any change in the form of the disease takes place it might occur

I In the rodents which cause the human outbreaks
II In the fleas which transmit the disease to man
III In the initial bubonic cases

I As to the first, the idea that the species of rodents involved in the epizootics might influence the character of subsequent epidemics is a very fascinating one Especially it has to be considered if a close relation does exist between epizootics in certain *wild* rodents and human outbreaks of *pneumonic* plague In fact a world wide study of the disease both in rodents and in man as undertaken by our staff within recent years has yielded many data supporting this view On the other hand in some countries where only ordinary rats are involved the incidence of lung pest is conspicuous also But before reaching any final conclusions the following points should be considered

(i) Though ordinary rats are sometimes found to be the original source of pneumonic outbreaks in many instances the local rodents were not involved the disease having been imported from outside by human agency, namely by travellers incubating the disease
(ii) How long has the disease existed in the local rats ? In some plague areas with rat epizootics pneumonic plague was frequent soon after the introduction of infection but became rare afterwards One might consequently suggest that the longer the infection continues among ordinary rats, the less chance there is for pneumonic plague to appear in man
(iii) Possibly when the epizootics for some reason receive a fresh impetus e g through re importation of infection or immigration of new animals their character and hence that of human plague may also change

It will thus be seen that different and often very intricate factors are at work A final judgment may only be reached if and when the conditions present in the different plague areas are investigated according to a uniform scheme

II Regarding the second possibility it seems improbable that the particular species of fleas have a bearing upon the character of the epidemics *X cheopis* is the sole culprit in some countries where pneumonic plague is frequent Further, it is debatable if the different fleas really play an independent part, or their apparent importance is due to the role of their hosts

III When we consider that apart from rare instances, primary pneumonic plague is not passed directly by the rodents to man but arises from human cases with secondary lung involvement it becomes clear that factors which help to mould such secondary pneumonic features deserve our serious attention

At the first glance it would seem likely that *frequency* of cases with secondary lung involvement ought to be of paramount importance for the rise of primary

neumonic epidemics A careful analysis of available data suggests, however hat the decisive factor is not so much this as the *degree* of lung involvement which ay develop in such cases This degree may be marked in only a few cases and in hese cough is frequent leading to the discharge of numerous plague bacilli in the putum I am sure that exact comparative investigations undertaken in this irection both in areas with and without primary pneumonic plague manifestation ould yield valuable results

If we ask ourselves what factors are primarily important for the development f marked lung involvement, mention must be made of

(i) an unusual susceptibility to respiratory diseases in general,

(ii) a lessened resistance to plague infection

That both factors are actually at work is proved by epidemiological bservations As an example we may point to the presence of severe secondary neumonia in travellers and the frequency with which pneumonic outbreaks riginate from such people

We may thus summarize the above discussion

(i) It seems probable that not one factor but a sum of different factors such s special character of the epizootics susceptibility to lung diseases in general or o plague infection etc is responsible for the rise of pneumonic plague outbreaks ach individual factor may not be present in every plague area where the pneumonic orm exists On the contrary it would appear that in the various localities a ifferent chain of circumstances influences the nature of the epidemics

(ii) Although stress must be laid upon intrinsic causes it is certain that xtrinsic factors indirectly contribute to the rise of pneumonic plague Climatic onditions may enhance the susceptibility of the lungs while bad social condi ons may impair the general resistance of the individual

(iii) In spite of the above explanations the difficult problem as to how pneu ionic epidemics arise is by no means solved I have merely pointed out certain actors which appear to be of primary importance Such can only be properly valuated when their influence in the various plague areas is investigated accord ig to a uniform scheme

(2) *Spread of Epidemics*

While extrinsic factors alone cannot account for the rise of pneumonic *pidemics they are instrumental for the spread* of the disease The factors at ork may be classified under three headings

(*a*) Climatic influences

(*b*) Social influences

(*c*) Influence of measures taken

It is obvious however that no sharp line can be drawn between the three roups Thus both climatic conditions and poverty may cause overcrowding nd lack of ventilation both ignorance and extreme cold may interfere with the anitary measures

There is also no doubt that the influences at work are of unequal importance in the various plague areas, with their different climate population, economic conditions and customs It is therefore not strange that the diffusibility of pneumonic plague varies so much The presence of many favourable factors or even a few powerful ones may foster the spread of the disease Vice versa, the absence of similar conditions tends to cut short a continuation of the outbreak

A few points deserve special mention

(a) Some authors not merely associate the influence of adverse climatic conditions with the creation of unhygienic conditions in the tightly shut and overcrowded houses, but also point to more complicated reasons Thus Teague and Barber assume a low water deficit of the atmosphere as present in cold climates to cause the droplets of sputum to float longer in the air and thus favour the spread of the disease

This theory is in my opinion negatived by two facts

(i) Pneumonic plague occurs not only in cold climates but also in warm countries where evidently the atmospheric conditions considered as essential by Teague and Barber, do not exist

(ii) Still more important is the fact that a mediate infection in pneumonic plague as presupposed by this theory, though perhaps occurring occasionally is rare, pneumonic infection being usually contracted in the immediate vicinity of the patient I will return to this question later on when dealing with the infectivity of the disease

(b) It is a characteristic feature of many pneumonic epidemics e g in Manchuria that either no secondary infection of rats occurred at all or that—when such resulted in rare instances—no epizootic followed Certainly in epidemics running their course during cold winter, a low flea rate and unfavourable biological conditions for the fleas might be responsible But such an absence of secondary rat infection was observed under other climates as well e g in Upper Egypt On the other hand it is claimed by observers in India (Punjab) that such a secondary spread of infection to rats may occur and lead to real epizootics accompanied by human bubonic cases The possibility of such rat infection must, therefore, not be under rated, and further attention should be paid to this important problem

(3) *Decline of Epidemics*

When dealing with the spread of pneumonic plague, we emphasized the value of the measures taken against the disease The object in view is not only a limitation of the spread of infection, but also a speedy termination of the epidemic Obviously the same factors which influence the steps taken to limit its sway are also at work in stamping out the disease With ample means at the disposal of the medical staff and a free hand to use them the good will and active co operation of the population will hasten the beneficial results Vice versa, insufficiency of means, prejudice and opposition will postpone them Another conditional factor

is the weather Outbreaks which start under unfavourable conditions (e g , cold, rainy seasons, etc) will tend to diminish in severity so soon as climatic conditions show an improvement This may be brought about directly by facilitating the anti plague campaign or indirectly by lessening overcrowding improving ventilation etc On the other hand severe conditions of the weather setting in after the appearance of an outbreak may considerably aggravate its course

It is thus evident that as in the spread of pneumonic epidemics, their decline also depends upon a combination of different factors, the relative importance of which varies in different plague areas

A much debated question is whether there may also be a tendency for the outbreaks to decline *spontaneously* Some believers of this possibility explain it by a lessening in the virulence of the *B pestis* Such an assumption is, however, not borne out by facts The bacilli passing directly from man to man, do not become less virulent, but on the contrary there is evidence that during a pneumonic epidemic their virulence is enhanced It might seem paradoxical to maintain a spontaneous decline of epidemics under such circumstances But the findings made in Harbin and Vladivostok at the end of the 1920 21 epidemic tend to solve this riddle most of the cadavers dissected at this period did not show the usual pneumonic foci but presented—though undoubtedly infected through the respiratory tract—only hyperæmic and œdematous changes in the lungs as well as marked septicæmic features We designated these cases as pulmonary plague, because, though anatomically similar to the septicæmic ones encountered in bubonic plague, they were ætiologically different from them the infection having entered through the respiratory tract These patients with *pulmonary* plague are undoubtedly less infectious than those with the usual features of plague pneumonia

There is no reason to assume that these *pulmonary* cases were caused by plague bacilli weakened in virulence Most of them were not seen clinically but found dead in the streets , their illness was probably short and they were thrown out of the houses before the search parties could detect them The only case we saw clinically died on the day after admission to hospital without showing any cough or expectoration Thus we concluded that these cases were probably due to an enhanced virulence of the *B pestis* developing through passage from lung to lung We assumed that through such repeated passage the invading organism finally became so virulent as to cause mainly *pulmonary* cases These—though very fatally infected—are comparatively non infectious, because the disease runs a rapid course and as a consequence the principal medium of infection namely, the cough is absent In other words our post mortem findings at the end of the 1920 21 outbreaks afford a scientific reason in favour of a spontaneous decline of pneumonic plague epidemics

D PATHOLOGY OF PNEUMONIC PLAGUE

Most authors agree that primary pneumonic plague is due to an infection through the lower portion of the respiratory tract A minority, however, take an

opposite view, assuming that it enters through the tonsils etc and reaches the lungs secondarily through the blood or lymph stream It appears to me that the evidence available both upon pneumonic cases and such with tonsillar lesions does not support this view I would like to point specially to an unique case observed by Jettmar of our Service, concerning a man who committed suicide in the early stages of pneumonic infection

Russian peasant, aged 31 suddenly fell ill with shivers on the evening of February 18th 1921 accompanied by headache and pains in chest Before daybreak he left his hut and was found soon afterwards hanging from the fence of his garden Owing to the head and neck being frozen no complete autopsy was performed The lungs were examined in situ and samples taken from them and spleen for histological examination

Macroscopically the right lung showed hepatization of the middle lobe with confluent pneumonic foci in the upper lobe and early engorgement in the lower lobe The left lung was not materially altered.

Histologically the alveoli of lungs were filled with blood serum and exudative cells Where the changes were early plague bacilli were found throughout the alveoli in the more advanced foci organisms were concentrated near the alveolar walls adjacent to small vessels

No plague bacilli could be seen in the capillaries these however showed general congestion but no thrombi Likewise other blood vessels were apparently free from bacilli But the organisms could be seen forming dense clusters in the lymph spaces of the adventitia and media At places they even reached the outer wall of the intima here proliferation inflation and desquamation of the endothelium were observed

The spleen showed inflammatory hyperplasia but *no plague bacilli could be seen anywhere*

This case well illustrates the fact, established also by other observers that bacilli generally appear in the blood after lung symptoms have already manifested themselves Indeed, a few cases are on record, where death had occurred before bacteriemia had developed

Koulecha the main advocate of the tonsillar theory, says that a final decision of this problem can only be reached by experiments and not by histological findings This evidence may now be taken

Strong and Teague experimenting upon a large number of monkeys, found in animals infected by spraying that the lesions corresponded exactly to those of human pneumonic plague the alterations in the fauces and cervical tissue were in practically all instances slight and obviously secondary in nature On the other hand monkeys which were infected in the fauces all died of plague septicæmia with or without bubonic infection of the cervical glands, that is in the case in which the infection was severe and the susceptibility of the animals more marked, they succumbed to septicæmia before cervical buboes developed In none of these instances was pneumonia present'

Analogous experiments were performed in 1925 by Wu Lien Teh and Jettmar upon tarabagans and susels We examined on one hand animals which succumbed to plague inhalation spontaneously and on the other those which had been killed in early stages of the infection In no case did we see primary foci in the fauces (tonsils) trachea or main bronchi Occasionally they were situated in smaller bronchi Most often, however the portal of entry was found in the deepest parts of the respiratory tract, the *bronchioli respiratorii* and the alveoli It may thus be

concluded that our experiments while following the line of Koulcha s wishes have produced exactly the opposite results this author expected In other words we are sure that pneumonic plague invades the body through the lower portion of the respiratory tract

E Infectivity

This well established fact is not only of theoretical interest but of great practical importance For it is evident that such a mode of infection may not be easily effected by merely coming in touch with contaminated objects Even the patients may be considered as comparatively harmless unless they cough or otherwise spray the infective material

These theoretical expectations have been fully confirmed by practical experience Only a few instances are on record where the evidence of a mediate infection is convincing or even suggestive Often children and aged persons are seen to escape infection This is certainly not due to their being immune but to the fact that they are not so apt to be in close contact with the patients as the middle aged Frequently simple measures of precaution apparently useless from a theoretical point of view have been found to save the lives of persons sharing the house or even the room of the sufferers because these helped to keep the contacts outside the range of the patients cough Therefore it may be safely concluded that in the same way that the transmission of bubonic plague depends upon blood sucking insects particularly the rat flea so pneumonic plague is conveyed from man to man by the cough of patients

Having ascertained that the real danger of pneumonic plague infection lies in the immediate range of a coughing patient we must further ask ourselves

(a) Whether a short contact of this kind is sufficient to cause infection

(b) Whether the infectivity of the patient is equal under all circumstances

Ad (a)—Infection through *short* contact undoubtedly takes place As a rule however there is a history of *prolonged* contact with previous cases and instances are on record where persons coming quite near to a patient for a short time remained unharmed

Ad (b)—Regarding the second question it has been firmly established that owing to the absence of cough and expectoration during the first stage of the disease the patients are then practically non infective Indeed it is the ideal of our preventive campaign to detect and isolate patients during this period which is usually of 24 hours duration

As soon as this first stage of the illness is passed the patients become more and more dangerous on account of a continuous increase of both the cough and the number of plague bacilli emitted by it

A controversial point raised by some observers especially French workers in West Africa is whether pneumonic plague is less infectious under warm than cold climates It would seem to me that the arguments brought forward in favour of such a lessened infectivity are not convincing Firstly it is doubtful whether

primary pneumonic plague is so frequent in French West Africa as sometimes assumed Apart from this, it is apparently not the infectivity of the individual patients so much as the diffusibility of the disease which is at variance Proximity to the patients within a certain radius is apparently fraught with equal risks under all conditions On the other hand, it is possible that the range of direct infectivity may somewhat vary under different climatic conditions It would be well therefore, if tests in this direction as performed by Strong and his collaborators in Manchuria, could be made in as many plague areas as possible I believe that such observations would reveal only gradual but no fundamental differences

F Value of Vaccines and Sera

(1) *Prophylaxis with vaccine and serum* —It is known that a pessimistic opinion as to the value of vaccine prophylaxis in pneumonic plague is entertained by many authors An exhaustive study of this subject shows however that this problem is not sufficiently elucidated to permit final conclusions While it is possible that further tests performed upon a large scale under rigid controls may produce better results than have been obtained hitherto I cannot help pointing to the great difficulties existing in this direction

The inoculation of vaccine is sometimes opposed on theoretical grounds it being maintained for instance that a negative phase follows which renders the inoculated more liable to infection I am led to believe that this danger is more imaginary than real In fact it appears that vaccination tends to prolong the incubation period It is likely, however, that immunity develops not at once but gradually and that it takes some days before full protection is afforded to the vaccinated Under these circumstances it would seem advisable to combine vaccination with prophylactic serum administration In fact it seems that the latter tends to confer quick though short lasting immunity and its combination with vaccine might be a hopeful method In the case of the personnel, exposed to infection throughout a long epidemic, it may not be wise to administer prophylactic serum, as anaphylactic symptoms may result when therapeutic doses become necessary later on For the ordinary contacts of the patients this objection does not hold Here, however, the high cost of such combined vaccination and the additional work for the staff have to be considered As already alluded to, prophylactic vaccination (and similarly serum administration) are apt to prolong the incubation period, so that the observation for contacts would have to be prolonged involving further expense Last but not least, it must be kept in mind, that even when no inoculations are practised the attitude of the population towards the medical staff is often hostile Should any of the inoculated develop plague this might be taken by the people to have occurred not *post hoc* but *propter hoc*, and the doctors be blamed for inoculating the disease under the pretext of fighting it I fully understand, therefore, why authors with great experience are sceptical in regard to prophylaxic inoculations in pneumonic plague epidemics and rely upon other means of prophylaxis It is

certain that the inoculations as available to day are no panacea, and further research is essential

(2) *Serotherapy* —The same may be said with respect to the therapeutic application of serum It is known that the prognosis of pneumonic plague is wellnigh hopeless I could collect less than 30 cases which presumably recovered from this disease among tens of thousands of records The fact remains, however, that most of the recovering patients and also a number of others who presumably suffered from marked secondary lung involvement, had received energetic serum treatment Certainly the best we can do at present for a pneumonic plague patient is to treat him as soon as possible with a potent immune serum Obtained—if feasible—from local strains in combination with a careful heart therapy and adequate nursing

G Concluding Remarks

In this rapid survey I have necessarily concentrated upon problems still *sub judice* omitting those which are already agreed upon I trust that I have not *created the impression of pessimism in regard to our chances of successfully com*bating pneumonic plague On the contrary, I believe that such epidemics will become less frequent and widespread for the following reasons —

(1) This form of plague is dependent upon epizootics and human cases of bubonic plague Whatever is done for the eradication of these will effectively help to stamp out the pneumonic form

(2) More attention is now paid to the pneumonic variety than in the past And the sooner this disease is recognized the easier it will be to check its spread A slow but steady change has also been noted in the attitude of the public towards the medical staff If they co operate or cease opposition, much quicker results will be achieved than heretofore

(3) Finally much may be hoped from the spirit of international co operation now prevailing as demonstrated by this meeting of experts initiated by the Health Section of the League of Nations There are several problems of pneumonic plague which can only be settled by common endeavours I trust that the day is not far distant when their solution will be firmly taken in hand

EXPERIMENTS ON THE TRANSMISSION OF PLAGUE BY X. *CHEOPIS* AND X. *ASTIA*

BY

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VARIOUS factors such as the climate, agriculture and trade, the facilities of communication, the number and habits of the people, the number and susceptibility of rats to plague and the number of fleas found on them have been indicated as the probable explanation of the susceptibility to or immunity from plague of a given locality. Of late years another factor concerned in the epidemiology of plague has been brought to light by the researches of Hirst. This investigator sent a collection of fleas from Madras and Colombo in 1913 to Rothschild who had recently identified a new species of *Xenopsylla*, namely, *astia*. Rothschild reported that they were all *astia*. Hirst (1913) therefore put forward the suggestion that the plague-free districts of the Madras Presidency owe their immunity to the prevalence of *astia* there. Later in 1923 and 1926 he supported this hypothesis by experimental evidence which tended to show that *astia* was a relatively less efficient vector of plague than *cheopis*, the latter in his opinion was the plague flea *par excellence*. He failed to obtain a successful transmission from rat to rat with *astia*. Considerable importance is attached to Hirst's discovery as affording a possible explanation of the immunity of certain regions from plague. Taylor and Chitre (1923) were, however, unable to demonstrate the difference found by Hirst in the ability of X. *cheopis* and *astia* to carry plague. In the opinion of the United Provinces Government the question demanded renewed inquiry and I was deputed to undertake the investigation.

This investigation lasted for about six months during which period fifty-two transmission experiments were carried out at Lucknow on the lines developed by the Indian Plague Commission. The details of the technique will be published in a subsequent communication. Attention may however be drawn to one or two points which are of interest. In the first place if the results were to be comparable strictly parallel experiments with *astia* and *cheopis* had to be carried out. To attain this end one set of both *cheopis* and *astia* experiments was started at the same time, the rats in each set being infected with the same spleen. Equal numbers of male and female fleas of the respective species were introduced in the cages at the same time. The protocol of one such set of experiments is given to illustrate this arrangement (Table I).

TABLE I

Protocol of a Set of Plague Transmission Experiments with X cheopis and X astia

Date	*Cheopis* EXPERIMENT			*Astia* EXPERIMENT		
	'X' Rat	*Cheopis* introduced	'Y' Rat	'X' Rat	*Astia* introduced	'Y' Rat
17th January, 1927	'J' 96 infected intra cutaneously with spleen of 'J' 89	4 M		'J' 97 infected intra cutaneously with spleen of 'J' 89	4 M	
18th January, 1927	Alive	19 F, 5 M		Alive	19 F, 5 M	
19th January, 1927	Alive	13 F, 8 M		Alive	13 F, 8 M	
20th January, 1927	Dead, plague		'J' 103 introduced.	Dead plague		'J' 104 introduced
25th January, 1927			Alive			Alive
28th January, 1927			Dead, plague			Alive
29th January, 1927						Dead, plague

M. = Male fleas
F = Female fleas

'X' = Rat inoculated with plague
'Y' = Test rat

Rats which have been used as test animals in this investigation are the natural hosts of both species of fleas *cheopis* and *astia* Before inoculating them with plague and introducing the fleas into the cages, it is obviously desirable that the rats should be freed from fleas Hirst gets rid of the fleas by combing the rats thoroughly A more satisfactory method, in my opinion is to dip the rats in petrol Williams (1926) has employed ether for this purpose Various methods have been suggested for the supply of accurately identified fleas which are required for the transmission experiments To Taylor, however belongs the credit of devising a method which is simple and practicable on a large scale Briefly stated, the method consists in the examination of the fleas in capillary glass pipettes under the low power of the microscope It is of vital importance that the flea should be absolutely motionless during examination If the flea lies with its head towards the capillary end, there is not much trouble The results of the experiments have been summarized in Table II

Out of 52 experiments in which X *cheopis* was used a successful transmission has occurred in 25 while under exactly similar conditions nine out of 52 experiments with X *astia* were successful These results support the view that X *cheopis* is a much more efficient vector of plague than X *astia* A further point of interest that emerges from a study of the table is the relationship of saturation deficiency with the experimental transmission of plague It will be noted that a higher saturation deficiency is associated with a lower percentage of positive results This is apparently due to the inimical influence of a high saturation deficiency on the duration of life of the flea This relationship however is not constant for in November with a saturation deficiency of 0 197 of an inch the percentage of successful transmission with *cheopis* and *astia* is 81 and 27 respectively whereas with a lower saturation deficiency of 0 124 of an inch in January the percentage of positive results is 46 with *cheopis* and 23 with *astia* In seeking an explanation for this discrepancy it may be pointed out that the number of fleas introduced has not been the same in all the experiments The number of fleas placed in the cage has varied from three to 53 The lowest number introduced which resulted in a successful transmission was nine In order to study the influence of the sex of flea in the transmission of plague experiments were made with the male and female fleas separately Out of four experiments in which only male *cheopis* was used a successful transmission was obtained in three while under the same conditions none out of the four was successful with female *cheopis* With *astia* one experiment was made and was successful with males and not with females These results lend further support to the conclusion arrived at before (1927) that males of both the species carry plague more readily than females under laboratory conditions In nature, however, this may not be the case because the male has a very short life apart from its host as compared with the female which lives much longer and is probably the more efficient transmitter In experimental transmission in the cages, on the contrary the food is close at hand and the fleas have not to pass any considerable time apart from their host

TABLE II

Plague Transmission Experiments with Madras and Jhansi Rats

Month	Humidity relative	Temperature (Fahrenheit)	Saturation deficiency in inches	Total number of experiments with each species	SUCCESSFUL EXPERIMENTS		PERCENTAGE OF SUCCESSFUL EXPERIMENTS	
					Cheopis	*Astia*	*Cheopis*	*Astia*
October 1926	66 2	77 2	0 278	5	1	2	20	40
November 1926	70 9	66 6	0 197	11	9	3	81	27
December 1926	71 2	61 6	0 165	10	5	1	50	10
January 1927	77 4	58 4	0 124	13	6	3	46	23
February 1927	66 0	64 4	0 214	9	4	0	44	0
March 1927	42 0	71 5	0 468	4	0	0	0	0
TOTAL		.	.	52	25	9	48	17

Another series of transmission experiments was carried out in the flea breeding cages These have been designated as continuous transmission experiments Two Jhansi rats were infected with plague One was placed into one cage together with a number of *cheopis* the other plague infected rat was introduced into another with the same number of *astia* Equal numbers of *cheopis* and *astia* were introduced into the respective cages at irregular intervals The inoculated rats on their death were replaced by another set of infected rats By the time the second set of inoculated rats had died a sufficient number of fleas had been introduced into the cages and therefore a good number of infected fleas was probably available for transmission This second set of infected rats was replaced by fresh healthy animals which on their death were replaced by other healthy rats and so on Successive and successful transmission was obtained with eight rats in the *cheopis* cage and with only two in the *astia* cage The second continuous transmission experiment was begun on the 22nd February 1927 Plague was carried to only one rat with *cheopis* and to none with *astia* The rat in the *cheopis* cage succumbed to plague on the 7th March During the first ten days of March the saturation deficiency had risen to 0 553 of an inch with the atmospheric temperature at 68°F From these experiments as well as those carried out in the double cages it may be concluded that at a saturation deficiency of 0 6 of an inch *cheopis* is not capable of transmitting plague to rats in the laboratory The limit for *astia* is lower being about 0 3 of an inch Brooks (1917) studied the influence of saturation deficiency and of temperature on the course of epidemic plague in India and concluded that plague epidemics are brought to an end when the temperature rises about 80°F accompanied by a saturation deficiency of over 0 3 of an inch In his opinion ' there is a critical saturation deficiency for each range of temperature At 80°F the critical saturation deficiency was 0 3 of an inch He remarks further At lower ranges of temperature a higher degree of deficiency is needed to suppress the epidemic while at higher temperatures a somewhat lower deficiency will suffice Experimental transmission of plague has been obtained with *cheopis* in the laboratory at a high saturation deficiency of 0 553 of an inch but the temperature was comparatively low viz 68°F

REFERENCES

Goyle A N (1927) *Ind Med Gaz* LXII No 6 June

Hirst L F (1913) *Jour Brit Med Assoc* (Ceylon Branch) Read 13th Nov

Idem (1923) *Ind Jour Med Res* X 789

Idem (1926) *Ceylon Jour Med Sci* I 157

St John Brooks R (1917) *Jour Hyg* Plague Supplement V 881

Taylor J and Chitre G D (1923) *Ind Jour Med Res* XI 621

Williams C L (1926) *Amer Jour Trop Med* VI 367

AN UNRECOGNIZED TYPE OF PLAGUE

BY

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THE term plague was applied by older writers as a general designation of various infective diseases that broke out into virulent epidemics Thus typhus and relapsing fevers the true plague and malignant types of the exanthemata were labelled as *black plague* either on account of external manifestations or from the character of the vomited matter or excretions e g coffee ground vomiting or hæmaturia etc It is probable that the septicæmic type of plague with large patches of cutaneous hæmorrhages as also the type about to be described in this paper were included in this category Modern writers have classified plague as bubonic pneumonic septicæmic and ambulant, but they have failed to recognize the cellulo cutaneous type because of its comparative rarity or its being considered as a complication of bubonic plague under the old designation of carbuncles Whilst bubonic plague has contributed about 93 per cent of all cases at the Bombay hospitals and the septicæmic and pneumonic types about two and one per cent respectively the proportion of cutaneous type has varied from four to six per cent in different epidemics, at times more evident whilst at others scarcely noticeable Moreover, it has been recognized that it is the most benign type of plague with the lowest rate of mortality

The average rate of recovery in over 800 cases has been about 37 per cent as compared with 26 per cent in the bubonic type I have had exceptional opportunities to study the beginning development and progress of this type and the following notes are a brief summary of the observations conducted and repeatedly verified over a series of epidemics It has been reported to me that during this year many more cases of the type have been observed than during the last few years Is it because plague having finished its cycle of 30 years at Bombay the virulence of the bacillus has become attenuated and more cases of the localized type have developed ?

Its development is dependent upon the peculiar property of the *Bacillus pestis* whereby its protoplasm or endotoxin is capable of setting up inflammatory and retrogressive changes of tissue elements, leading to progressive local necrosis

Klein(1) says in reference to other organisms (but equally applicable to this bacillus) that the necrosis following typical symptoms of inflammation is the result of the local action of the bacilli, and so long as they are in sufficient numbers and of sufficient virulence and so long as their multiplication proceeds the necrosis of the tissues spreads into larger and larger areas That is well exemplified in the case of large necroses often observed in plague

Definite lesions at the point of infection in plague exist in about eight to ten per cent of cases in the form of phlyctenules, pustules bullæ and umbilicated bullæ It is from the two latter that this type originates If the bulla is cut open or bursts of itself and the central spiral core of the sweat gland removed its point of attachment to the base appears as a small dark spot It gradually increases in size until the whole of its base is encroached upon and obliterated by a dark eschar This continues to spread at the circumference from day to day until a period is reached when the medium suitable for further multiplication of the bacillus becoming exhausted a line of demarcation appears The eschar assumes a dark greenish hue feels cold to the touch is almost hard and leathery and depressed in the centre as if firmly bound down to the tissues underneath Its diameter may vary from one inch to even 13 inches Its spreading margin somewhat higher is conterminous with the surrounding skin which becomes converted into a hard angry inflamed areola studded with minute vesicles and often small and secondary necroses are noticeable at some distance from the periphery through infection from the parent necrosis when it happens to be extensive Multiple umbilicated bullæ are also occasionally met with on different parts of the body surface each giving rise to a small necrosis If a little fluid be examined from the margin it invariably shows a pure culture of the bacillus The destructive process if limited becomes a comparatively benign form of plague as said above to which I have applied the term cellulo cutaneous plague The buboes if present—they are not invariably so—are secondary and owing to the slowness of the process the course of the disease is prolonged from two to three weeks instead of the normal period of eight to ten days It is probable that recovery is determined as Pescarolo and Quadronet(2) say in relation to Eberth s bacillus in typhoid fever by the behaviour of *Bacillus pestis* in the tissues in relation to the processes of infection and immunization which differs as we have already seen from what it is when confined to the circulation and in its conflict with the cellular elements of the tissues immunizing bodies are produced which bring the infective process to a rapid termination Should the necrosis become extensive as in some cases it does involving as much as 60 to 90 square inches of the body surface the infection becomes generalized and the patient succumbs through septicæmia Once the line of demarcation has formed it is not difficult to remove the entire slough *en masse* leaving behind a saucer shaped depression in the subcutaneous tissue (often exposing the deep fascia and muscles) of gradually increasing depth from the periphery to the centre, filled with shreds of necrosed tissue pus and blood it gradually assumes a healthy aspect under

appropriate treatment. It is marvellous to observe how large areas rapidly heal up without the necessity of skin grafts. Such necrosis has been observed on the scalp, neck, face, back, chest, arm and forearm, shoulder, the abdominal wall, lumbar and gluteal regions, over the mammary gland, the vulva, and on the scrotum, the thigh, leg and foot, in fact wherever such umbilicated blisters might be situated. The destruction of the soft tissue is often enormous and large cavernous cavities exposing the bones of the face and head, involving exfoliation of the periosteum and as also the cartilages of the ear, the eyelids, and the nasal bones. The upper and the lower jaws may be exposed when the process is confined to the head, neck and face; the ribs may become exposed on the side of the thorax, and the testes if the scrotum is involved; sometimes the abdominal walls are destroyed and a large cavity exposing the intestines is to be seen.

The clinical features are confined mainly to the local manifestations, its progress being marked by a slow and steady advance from day to day until the *line of demarcation forms*. The presence of buboes in direct lymphatic communication with the necrosis is dependent upon its extent. If the necrosis is small and less than a rupee in size, the buboes are more pronounced and the clinical phenomena almost identical with those observed in the bubonic type. Should it be larger—four to six inches in diameter—the buboes are of subordinate significance, inasmuch as the invasion being strictly localized, the general disturbance of the system is not so great and the result favourable. When the primary necrosis is at all extensive and oversteps the above limit, there are more chances of septicæmia developing, or again the patient may succumb through secondary infection or prostration even after its excision.

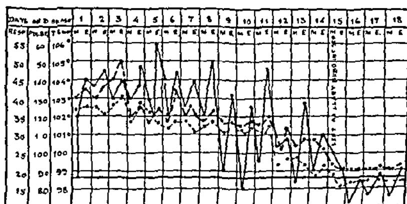


Chart of a normal case of plague of the cellulo cutaneous type

The accompanying chart illustrates the temperature curve of a case in which two necroses existed under the left costal margin, together with a bubo in the left axilla; the latter suppurated and was incised on the fifteenth day of illness. The course of the affection is seen to be prolonged to a fortnight, and the temperature curve is quite dissimilar to that in the bubonic type.



Fg 1—A s nall nec o on t e ba k the lumbar re on w th se on lary buboes in Sen pa s t a gle eco ery



Fg 2—Woun ls f om two necroses one on the scalp an l the other on the s de of t e neck in the process of c atr zat on



Fg 3—A large necros nvo v n the forehead and s de of the face ove the ten po al zygomat c malar and nasal reg ons with des uct on of the upper and lower eyelids reco ery

The accompanying illustrations demonstrate the necroses in different cases

The above observations relate to the primary cellulo-cutaneous type of plague There exists however, a *secondary type* which fully corroborates the genesis of the type as above described Occasionally a deep seated bubo appears to enlarge upwards, becomes adherent to the skin over which there develops a blister just like the initial blister of the primary type The blister bursts a dark eschar forms at the base and it gradually extends over a considerable area forming a large necrosis When it is eventually removed, the bubo is found adherent to its under surface a large and deeper cavity being left at the site than in the primary type

I have ventured to place the above facts before you after observations extending over 27 epidemics of plague at Bombay in the hope that clinical observers would not overlook it and it may be included with the hitherto three principal types already acknowledged It is not possible to forecast what process of evolution in the virulence and characteristics of the *bacillus pestis* may take place in the future May it not evolve a milder type of the disease, though up to the present it exhibits all the malignity of the older epidemics without any attenuation of its virulence ?

REFERENCES

(1) KLEIN (1900) *Brit Med Jour*, Aug 4th
(2) PESCAROLO and QUADRONTI (1908) *Ibid* Nov 14th

THE PERPETUATION OF PLAGUE AMONG WILD RODENTS *

BY

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TABLE OF CONTENTS

A INTRODUCTION

As in other epidemic diseases one of the most fundamental and withal fascinating problems in plague is the question as to how infection is preserved and propagated from season to season, often without the presence of human cases and manifest rodent disease

In this report I intend to deal with wild rodents, but, for the sake of comparison it may be worth while to touch at first upon the situation as it affects domestic rats

In their ' Summary on the Ætiology and Epidemiology of Plague '(1) the Indian Plague Commissioners come to the conclusion that the periods between the epidemics are bridged over by cases of *acute* rat plague, the epizootic being kept in check by —

(*a*) a high mean temperature prevailing,
(*b*) a diminution in the total number of rats together with an increase in the proportion of immune to susceptible animals and, finally,
(*c*) a diminution in the number of rat fleas

* In the preparation of this article I have received much assistance from Dr Robert Politzer of our staff To him I wish to accord my best thanks

In an earlier contribution(2) the Commissioners had already refuted the theory that cases of chronic (or better resolving) rat plague are important in the propagation of infection Among the reasons given are —

(a) It is unlikely that rats might contract acute plague by feeding upon the carcasses of chronic plague animals

(b) There is no evidence that resolving plague changes might 'light up' resulting in bacteremia and thus rendering the rats dangerous

The Commissioners therefore conclude that these chronic plague rats inasmuch as the bacilli are shut up in abscesses where fleas cannot possibly get at them are *per se* of no importance in spreading the infection '(1)

Since this statement was issued in 1908 we find again and again reference to a possible role played by chronic plague rats To our knowledge no satisfactory evidence has been brought forward to support such claims and it may still be maintained that resolving rat plague is a sign of past infection rather than an active factor in the propagation of the disease On the other hand it is probable that sporadic cases of acute rat plague are not the only ones which keep the virus alive during the off seasons Recent investigations especially by Williams(3) suggest that 'carriers' of plague exist among the rats which though apparently surviving for a prolonged period display bacteremia and may therefore spread infection through their fleas A further possibility is that rat fleas which under suitable conditions are apt to remain alive and infective for a considerable time may help to preserve the virus

Turning now to the wild rodents we find that more or less satisfactory evidence is forthcoming for a few species only Among those hitherto investigated blood sucking ectoparasites specially fleas have been found to be the principal if not the only means of transmitting plague Therefore animals with bacteremia are an essential link in the propagation of this infection

B THE GROUND SQUIRREL OF CALIFORNIA (*Citellus beechyi*)

Two factors favouring the spread of plague among ground squirrels are —

(a) Absence of a seasonal prevalence heavy infection being noted in winter as well as in summer(4)

(b) Restriction of æstivation or hibernation to adult animals principally Though this tendency to æstivation or hibernation (or probably a combination or both) is noted in ground squirrels adult animals are mostly affected(5) The young animals suspected by Harrison(6) to be most susceptible to acute plague keep awake throughout the year

An important means of staying the spread of plague among squirrels is the immunity usually developing in regions where the disease has prevailed for several years McCoy(7) in an early contribution on this subject says that this evolution may mean a gradual extinction of the disease or it may indicate that this partially

resistant race of rodents will if not vigorously attacked perpetuate the disease for many years' Now there is no doubt that the infection tends to perpetuate rather than disappear The immunity prevailing in endemic localities is not absolute as has been shown by the instructive experiments of McCoy(1) upon squirrels from plague affected and plague free zones respectively —

Squirrels from	Total used	Died		Killed	
		Acute plague	Sub acute plague	Residual bubo	*No lesions*
Plague zone	16	1	3	3	9
Plague free zone	19	8	9		2

It can thus be seen that sub acute and even acute plague though rare, were not entirely absent among squirrels of the first category A similar variation *in the lesions is also seen in naturally infected squirrels Not only those with acute* disease but also some of the sub acutely affected animals show bacteremia and are therefore infective Moreover it is claimed by Rucker(8) that even in regions where the disease has existed for a few years and the majority of animals present non acute plague small areas will be found, e g, isolated valleys where the acute form preponderates For all these reasons it is not difficult to understand why plague persists among the Californian wild rodents

C THE SOUTH AFRICAN WILD RODENTS

A very rapid type of infection is noted among the wild rodents involved in South African epizootics As far as our present knowledge goes chronic plague does not exist among either gerbilles or multimammate mice The evidence in regard to carriers is scanty, only one Namaqua gerbille and one Karroo rat respectively found in one and the same locality suggesting such a condition It is evident therefore that once plague infection is introduced in a locality many animals will succumb quickly little fuel remaining to keep the virus alive A satisfactory explanation for this situation has been given by Pirie(9) who proved that plague infection could be maintained in a scanty rodent population by flea transmission In a series of well planned experiments he demonstrated that the fleas could carry over the infection for a period of three or perhaps four months He reasons thus —

The mechanism of the persistence of plague in the wild rodent reservoir is therefore not too difficult of explanation even when the rodent population is scanty When the rodents are few in number the disease would of necessity be of a quiet smouldering type through frequent failure of the necessary contacts to be established but a rodent infection every here and there at two monthly intervals would

be sufficient to keep the disease alive As the number of rodents increased and the fleas presumably increase *pari passu* so would contacts be more easily made and the number of cases increase

Whether increase in numbers to a high level is in itself sufficient to bring about the recurrence of definite epizootics every three or four years and still more markedly every ten or eleven years in accordance with Elton s theory, or whether some extra factor possibly a widespread climatic one must also come into play to allow of a flareup from the smouldering stage to that of the raging fire must be left for future observations to settle

D THE SOUTH RUSSIAN RODENTS

As in South Africa so in South Russia several species are known to suffer from plague Only a few are of fundamental epidemiological importance namely the small susliks (sisels) on the one hand and domestic and wild mice on the other An interesting point is that no regular transition of the infection from the susliks to the mice or vice versa exists so that the epizootics run an independent course in each group(10)

As far as we know the problem of how plague is perpetuated among the *mice* has not been studied in detail Chronic affections seem to be present in them(11) so it may be assumed that some immunity does develop in the course of the epizootics Among the domestic mice carriers have been observed as well *B pestis* growing in abundance though no macroscopic signs are noticeable (12) No doubt plague is constantly transmitted among the mice and not caused by periodical re infections from the gnawing of human plague corpses(13) It seems safe to assume that the epizootics among these non hibernating animals occur in the same way as among domestic rats

The *susliks* lie in another category since prolonged hibernation is an obligatory feature among them As in the case of mice it has been assumed in the past that plague is not constantly present in susliks infection being contracted after the hibernation period from plague corpses But the systematic work of Nikanoroff and his school has lately shown that such an unusual assumption is unnecessary

Nikanoroff(10) has suggested an influence of the seasons upon experimental suslik plague —

Tests were made in this direction by infecting fortnightly from the middle of June batches of 30 susliks with cultures of the same origin and uniform virulence Four series were made with subcutaneous injection In the first most animals succumbed quickly to acute plague while in the other three series the disease displayed more and more a slow evolution Thirty days after infection there still lived in the first group 0 , in the second group 5 in the third group 5 , in the fourth group 18

These results have been supplemented by observations of Gaiski(14) under taken throughout a whole year He infected subcutaneously 242 susliks in 27

batches, using for each successive series a strain isolated from the preceding Seasonal differences were noted in two directions —

(*a*) The mean length of illness varied reaching its minimum (3 days) in June and maximum (25 days) in winter

(*b*) While in June and July 100 per cent of the animals displayed bacteremia only 60 per cent were so found in winter and 40 per cent in March The other animals suffered either from pure local plague with bacilli confined to the site of infection or from a transitory form (bacilli in the organs but not in the blood) The incidence of local plague was highest in winter (30 per cent)

Of special interest are Gaiski s results with hibernating animals Of 30 such susliks—

awoke and succumbed after 2—22 days (average 8 days)	21
were killed after 15 and 35 days after infection respectively	3
succumbed after 15—138 days	6
TOTAL	30

Interesting details of the above experiments are —

(*a*) Of the three animals killed two (15 and 35 days after infection respectively) were well nourished and showed plague bacilli at the site of infection but not in the blood or organs In the third (killed after 15 days) bacteremia was present

(*b*) Of the three animals dying after 138 120 and 96 days respectively at the physiological end of hibernation at least one had passed through a stage of bacteremia At post mortem *plague bacilli were present* both in the internal organs and at the site of infection in the other two animals only in the local abscesses

Later researches by Golov and Joff(15) indicate that the suslik fleas have probably a considerable *share in keeping the virus alive* —

They prove that the fæces of infected fleas may harbour plague bacilli for a considerable time (observed up to 79 days at a temperature of 7°C to 10°C and 92 per cent humidity) though they were allowed to bite an infected animal only once and afterwards healthy ones

Infected fleas which were afterwards kept starving at temperatures corresponding to those of the burrows in winter survived up to 206 days and yielded at death living and virulent plague bacilli Golov and Joff also showed that suslik fleas kept in test tubes at such temperatures remain active and able to feed upon hibernating susliks Similar observations were also made under natural conditions

Suslik fleas can stand low temperatures (down to —25°C) and prolonged starvation in empty burrows (up to ten months)

It is thus seen that conditions for preserving the plague virus among the rodent population are almost ideal On the other hand, as pointed out by Gaiski, the seasonal changes of susceptibility may protect the species from extinction through acute plague even though no specific immunity develops

E THE ALPINE MARMOT (*A marmotta*)

These rodents though perhaps in former centuries playing some part in the spread of European plague have now to be classed among the experimentally susceptible ones They are, however of considerable interest to us in that beside being close relatives of the Siberian marmot successful hibernation experiments have been performed upon them Dujardin Beaumetz and Mosny(16) injected *B pestis* subcutaneously into three European marmots One non hibernating animal died in 2½ days The other two which hibernated succumbed after 61 and 115 days respectively showing no local reaction or buboes but foci of chronic pneumonia in which plague bacilli were present in enormous numbers These French observers consider this as sufficient proof that the tarabagan of Siberia is a reservoir for plague the virus being held in abeyance during winter Though this conclusion looks at first radical and rash it seems to be quite justified by our latest experiments upon the true Siberian marmot

F THE TARABAGAN OR SIBERIAN MARMOT (*A bobac*)

(1) *Introductory remarks*

The Siberian marmot like the susliks of South Russia undergoes prolonged hibernation Most authors agree that plague stricken animals do not seek shelter in the burrows but stay out and die in the fields Such rodents have been found on the surface long after the onset of the hibernation period having either not slept at all or got up again with the development of symptoms

For these reasons it is doubted by several observers if the virus is permanently kept alive in the tarabagan They contend that in autumn a thorough separation takes place between healthy animals which retire to the holes and sick ones which remain outside to die Different theories are conceived to explain how the tarabagan population becomes again infected in spring As in South Russia it was thought that the animals might contract the disease by gnawing human plague corpses This hypothesis is not only intrinsically weak but disproved by the fact that no importation of human plague takes place at all as was pre supposed It seems also unlikely that infection could be preserved in the dark and moist burrows of the tarabagan and propagated through animals searching for mates or other wise chancing into such holes A theory maintained by Sukneff(17) deserves attention According to him the reservoirs of plague are not the tarabagans but certain species of small rodents Among his contentions are —

(a) The tarabagan does not suffer from chronic plague

(*b*) Only healthy animals hibernate, whereas sick ones remain outside and die off

For many reasons we have never agreed with Suknеff(17) Though severely sick animals may no doubt stay out we have been able to show experimentally that those developing the disease hibernate in much the same way as healthy ones Regarding chronic plague in tarabagans we have supplied evidence of its existence in laboratory animals The question whether chronic plague exists in naturally infected tarabagans does not seem fully established Some of the morbid changes seen by us in 1923 appeared suspicious in this regard but our latest investigations show that it is not always easy to interpret such findings definitely However as already mentioned in the foregoing pages the presence of chronic plague is certainly not a *sine qua non* for the perpetuation of plague in a rodent species One is almost tempted to reason that the presence of chronically affected animals in other words the development of some immunity in that species complicates the question as to how the virus is kept alive Be this as it may it is easy to explain how plague is propagated among the tarabagans during the *warm* season without their being wiped out For these rodents living in families as a rule do not stray far away from their burrow but keep within its reach so as to escape danger It seems probable therefore that the disease among them bears usually a familial character On the other hand the infection certainly creeps slowly from burrow to burrow and then from settlement to settlement In this an important role is played by very sick animals which are seen to stagger about aimlessly and may shed some of their parasites on the way The latter will seek new hosts as their old ones die off Chance meetings may also take place among animals of different families e g during the mating season in case of danger etc

(2) *Earlier hibernation experiments*

The pivotal point of our problem is therefore what happens to the virus during the long *winter* Dujardin Beaumetz and Mosny s experience suggestive as it is cannot be taken as a valid proof that the *B pestis* is preserved in the Siberian marmot throughout the hibernation period Likewise our former (1922 23) winter experiments upon tarabagans(19) though showing that infected animals may continue to sleep and succumb to infection considerably later than those infected in summer were not wholly satisfactory For in order to obtain as much preliminary experience on hibernation as possible we adopted the following lines in tackling the problem —

(*a*) Different methods of infection were chosen so that comparisons could be made among small sub groups only

(*b*) The animals were daily handled in order to have their temperatures taken In all probability this often perhaps inevitably hastened their death

(3) *Plan for new hibernation experiments*

To eliminate errors, we approached the hibernation problem again and after careful consideration formulated the following plan —

(a) To extend our programme over two winters —

(i) In 1926 27 we waited for the spontaneous death of the test animals handling them as little as possible

(ii) In the winter 1927 28 we propose to kill some of the infected animals at regular intervals in order to study in more detail the manner in which the virus is preserved

(b) To infect the animals with doses of uniform size by pricking the paw choosing the inner aspect of the left hinder leg (Prick in the tail would have been more satisfactory in certain respects but in that case we would not be so certain of the bubo localization)

(c) To infect batches of two tarabagans fortnightly With each batch a guinea pig was percutaneously infected with the same material in order to confirm its nature and virulence

(d) To begin the experiments with our strain 'V'* and to pass it as far as possible directly from tarabagan to tarabagan (Failing this a culture from the guinea pig of the preceding batch was used)

(e) To house the infected tarabagans in the unheated outbuilding used in our former hibernation experiments (This plan was modified in the course of the work because a few animals seemed to die prematurely of unspecified lung or intestinal disease due perhaps to the extreme cold Hence from the third experiment onwards only one animal of each batch was kept in the outhouse the other in the warm plague room of the laboratory The evolution of infection did not seem to be affected by this difference in the temperature †)

(4) *Experiments in winter* 1926 27

In the winter of 1926 27 we infected altogether nine guinea pigs and 16 tarabagans (14 hibernating) Little need be said about the former With one exception they all died within three to seven days mostly on the fifth day of acute plague yielding typical cultures The exception was the guinea pig of the first batch which succumbed to plague on the eleventh day That the initial culture was virulent is proved by the fact that one tarabagan of this batch which did not continue to sleep died after six days of acute plague A guinea pig infected with material from guinea pig 1 also succumbed after five days to the disease Hence the lengthy survival of guinea pig 1 must have been due to individual resistance or some untoward incident

* This is a strain of tarabagan origin, which has since 1923 been repeatedly passed through laboratory animals It has high and stable virulence

† A table showing the temperature of the two rooms will be found in the Appendix

Of the 14 tarabagans hibernating at the time of infection (winter 1926 27) —

	Number	Condition after infection	
Died after 2 days no plague	1	Continued to hibernate	
Do after 5—19 days with signs of manifest plague with bacteremia	6	Slept fairly well	1
		Do interruptedly	4
		Awoke	1
Do after 22—60 days no plague	3	Continued to hibernate	2
		Slept interruptedly	1
Do after 28 and 48 days respectively with signs suggesting residual plague	2	Continued to hibernate	
Do after 88 and 130 days respectively i e a few days after awakening at the normal end of hibernation with signs of local and bacteremic plague	2	*See text*	

One striking feature of the above summary is the great variation in the results obtained It seems an open question if the same holds true in nature Try as one would one could not imitate the undisturbed quietness low but perhaps only slight oscillating temperature etc which reign whenever the tarabagans sleep in their natural habitat Hence it is more than probable that a certain percentage of our experimented animals died prematurely while others would have survived under natural conditions Nevertheless valuable conclusions can be safely drawn from the experiments These results may now be discussed in detail

(*a*) *Animals showing no signs of plague at post mortem* —Of the four tarabagans composing this group one showed broncho pneumonic foci as confirmed by histological examination, two had sub pleural petechiæ and had presumably succumbed to a lung process the fourth, dying two days after infection displayed signs of an acute enteritis In none of these animals were any macroscopic changes noted at the site of infection or in the inguinal glands In one instance a few non characteristic bacilli were seen in smears from the spleen while in the pneumonic case such were present in preparations from both spleen and lung All cultures were sterile

It is possible that an exhausting experimental and histological examination would have yielded some traces of plague infection Because of our plan to make a systematic search for such next winter we thought it wise not to spend too much time and energy upon chance findings It is therefore difficult at present to draw any final conclusions in regard to this group However, some of the hibernating tarabagans may escape *infection or overcome it*

(*b*) *Animals showing signs of local (probably residual) plague* —The findings in the two animals under this group point, perhaps in the same direction Their protocols are —

Tb 3 *a* infected 3rd January, 1927, died 31st January, 1927 i e, after 28 days having hibernated well throughout

P M —Big well nourished animal

No cl anges vis ble at s to of inf ct on In tle left ingu nal reg on one gland hyperæmic and increased to s ze of half pea a otl er of about same s ze f lk l w tl caseous matter Lu s pink notl ng abnormal I er somewhat enl r ed dark brown

Spl en sl ghtly e larg l l t firm Bl d l r full (no pelvic bubo)

B ological examinat n

Im sm ars from the i gu al gl nds a f w susp ous ba ter a S ears from tl e l ea t blood show a f w fa t b polar-sta ed bacill from spleen doubtful Cultures from nternal organs n gat ve Gu nea pig nf cted percutaneously w th mater al from the glands survived.

H s logical exa m nat on *

(') Caseating gland Capsule mu h th ken l w th l ucocyt c nfiltrat on Extens ve destruc t on of lymph at c tissue only remna ts of wl h are to be s en among necrot c masses Bacterio log al dia o ses difficult ow n to presenc of a or ous mass of granul r d bris no d finite bac ll seen perhaps i volution forms present

( ) Hyperæm c gland Capsule tl kened and nfilt ated Lympl t c t ssue much engorged numerous depos ts of a brownish p ment s gl b polar stained ba lli n the m lulls and near l lus

Tb 5-a nfected 1st February 1927 hed 1st March 19 7 t after 48 days having hibernated well throughout

P M —Large con ested area at s te of u fection In u nal gl nds on ft s le somewhat enl r ed but not congested Lungs pink w h small hyperæm c areas o surface Spleen not enlarg d but sl ghtly s fter than normal. Bladd r as in Tb 3-a

B t riological ex ation

C ltures from nter al or ans ster l Gu n a p nf ted per utan ously w th mat ial fro n the s te of inf ct on surv v d

His log al xam n ion

(i) Area of local reaction No marked con stion In tl e subcutaneous t sue hæmo l s s of va y ng xtent noted with s ght leucocyt c u filtrat on at places Th latter und r h l power show d scanty b polar stained bac lli

( ) Ing nal glands No mark d cl an es f anyth n the trabeculæ somewl at enl ed Caps le of gland is undoubt dly tl ke l and shows cell prold ratio b t no l u o t o infiltrat on

( ) Lu At yl ces gro ps of al eoli fill d w t a ellular exudate mostly erythrocytes (red l epat zat on) No pla ue ba or oth r n ro organ sm s

While there is little doubt that a few plague bacilli still persisted in these tara bagans (one at the site of infection and tl e other in a regionary lymph gland) it is

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* For our h stolog cal examinat ons we used, bes des Hemalum-eos n mostly Kossel s method of sta n g

10 c cs conc aq sol Meth Blue (Hoechst) is diluted in 100 c cs distill d water

To th s add 30 drops of 5 per cent sol of Sod Carbonate cryst) S x c cs of 1 per cent aqueous Eosin A extra (Hoechst) is now gently a lded wh l shaking so as to prevent formation of sed ment S lut on must be prepared and filtere l imm d ately before use

Stain sections n th s solution fo 10 to 30 minutes D fferentiate in dil acet c ac d (1 drop gla al l l t distilled water) until p nk colour ap pears n parts Pass throu h 96 per cent tl en al olute alcohol until no more blue com s out Then pass throu h xylol and mount in cedar wood o l

difficult to ascertain their true significance We shall later bring evidence to show that plague bacilli introduced during the hibernation period may *remain at the* site of infection cause certain changes there and finally lead to general infection when the animal awakes Naturally the question arises whether the findings in the two tarabagans above do not constitute such local deposits of bacteria which would have led to manifest plague had the animals survived until spring This *possibility will have to be affirmed or negatived by our investigations next winter* Up to the present we are inclined to believe that the changes seen in tarabagans 3 *a* and 5 *a* are the result rather of a successful struggle with the invaders and that the few bacilli still remaining would have eventually disappeared had the animals not died prematurely

(*c*) *Animals dying of manifest plague during the hibernation period* —Only one of the six animals of this group continued to hibernate fairly well after infection, four slept interruptedly while the fifth was up throughout the six days of its illness These observations are not easy to explain Though there is undoubtedly much reason in the contention of authors like Gaisly that infected hibernating rodents wake up because symptoms of plague develop in them we doubt if this always holds true Possibly in some instances the disease runs a quick course because the animals are disturbed on account of the artificial conditions in which they are kept

I proceed now to a detailed description of the more important changes —

(*i*) *Local reaction and bubo* —Even the few tarabagans in this series seem to fall into different groups On the one hand we had a case of purely 'septicæmic' plague without local changes (Tb 1 *a*) on the other, animals with a somewhat prolonged course of the disease and sub acute changes characterized by the presence of suppuration (Tbs 4 *b* and 5 *b*)

Tb 7 *b* succumbing on the 19th day after infection though belonging to this group did not show such marked gross changes It resembled macroscopically the animals 6 *a* and 6 *b* which displayed signs of acute plague thus standing between the two just mentioned groups

With the exception of Tb 1 *a* (which was not investigated in this respect) positive *bacteriological* results were obtained in each instance from the site of infection and bubo

The macroscopic findings were generally confirmed by *histological* examination

Tbs 6 *a* and 6 *b* show very acute changes at the site of infection and in the bubo At the former leucocytic infiltration and hæmorrhages are noted in addition to much congestion Plague bacilli ... nfiltra
rrhage
in big
ocytes

Peri adenitis which was not well marked macroscopically, is made evident by the microscope being characterized by much congestion leucocyte infiltration and even hæmorrhage at places

Table showing the macroscopic findings of this group

Tb No	Died days after infection	Local reaction	Bubo	Liver	Spleen	Lungs	Other organs
1 a	6	None	None	Fat infiltration. Sub-capsular hæmorrhages	Sl. enlarged and softer	No marked changes	Hæmorrhages in mesenterium. Serosa intestine congested.
4 b	12	Marked. Suppuration at places	Size bigger windsor bean. Caseous matter on section at places	Enlarged congested	Swollen and soft. India tint nodes	Sub-pleural petechiæ	Kidneys congested
5 b	10	Abscess size half pea	Size over windsor bean. Some suppuration	Small nodes and hæmorrhages size lentil all over	Much enlarged. Nodes up to size lentil	Some areas of congestion	Omentum adherent to spleen congested. Stomach mucosa congested. Small intestine at places congested. Mesenterial glands enlarged and congested
6 a	6	Hæmorrhages and indistinct infiltration	Small. Inguinal glands, right side enlarged and congested	Congested. hæmorrhages below capsule	Not markedly changed	No marked changes	Hæmorrhages in omentum
6 b ..	5	Infiltration size two peas	Size two peas. Marked periadenitis with hæmorrhages	Fat infiltration. Sub-capsular hæmorrhages	Much enlarged. Softer	Pale. Fine sub-pleural hæmorrhages	Hæmorrhages in omentum. Tubes and ovaries congested
7 b	19	Infiltration size two peas	Size windsor bean. Œdema of abdominal subcutis	No marked changes	No marked changes	Pale	Hæmorrhages in omentum and mesenterium

N. B.—Diagnosis in case 6 b confirmed by smears and cultures; in all other instances also by experiment.

The local changes in animals 4 *b* and 5 *b* are different from those just described Hæmorrhage is absent and instead of the more diffuse infiltration with leucocytes one sees more or less well defined agglomerations of such In Tb 4 *b* some reaction seems present in the surrounding connective tissue, where at places marked congestion and cell proliferation are noted Plague bacilli are pretty numerous often forming clusters in the abscess like formations In the buboes there is no hæmorrhage and less marked congestion than in the foregoing cases Smaller and larger caseating areas are present Plague bacilli are quite plentiful in bubo 5 *b*, where they are mainly arranged in clusters; in Tb 4 *b* they do not appear so numerous and involution forms are met with Peri adenitis is quite marked in this case though no hæmorrhage is noted, the capsule of the gland is apparently involved In animal 5 *b* changes round the gland are not conspicuous, some alteration of the capsule is noted

That the gross appearances in Tb 7 *b* were not so typical is explained by the fact that in this case hæmorrhages and some diffuse leucocytic infiltration are present at the site of infection But here also one notes under the microscope abscess like formations of leucocytes Again reaction on the part of the connective tissue has taken place, it would seem that the latter tries at places to encapsulate the abscess like formations or to penetrate into them Plague bacilli are quite numerous but not often arranged in clusters In the bubo there is marked congestion, and hæmorrhages are noted at places in addition to some caseation Plague bacilli are numerous, occasionally in groups, but on the whole do not seem so plentiful as in the acute cases The capsule is moderately thickened with some leucocytic infiltration and cell proliferation The tissues round the gland are congested and show leucocytic infiltration which is perhaps not so marked as in the animals succumbing quickly

(ii) *Liver and spleen* —In every animal of this group positive bacteriological findings were obtained from liver and spleen The morbid changes correspond in general to those observed in bacteræmic diseases, especially plague, sometimes the absence of marked lesions was conspicuous (*see* tabulation) Only two cases with peculiar features deserve special discussion —

(α) *Tb 4 b* —The spleen of this animal showed *indistinct nodes besides much acute swelling* Histological examination confirmed the presence of large and small areas where lymphocytes and leucocytes, at places mixed with red blood corpuscles, are embedded in uniformly contrast stained, necrotic tissue Plague bacilli (usually single) are fairly numerous within such areas At other parts, especially the periphery of the necrotic nodes, they are seen in enormous numbers forming clusters and nets Even within the Malpighian bodies some *B pestis* are met with, especially at spots where a little hæmorrhage seems to have taken place

(β) *Tb 5 b* —Here marked appearances of 'nodose' plague were noted in both liver and spleen Histological investigation reveals severe alterations in the former consisting of marked congestion and infiltration of the liver cells with fat globules, at places more or less extensive hæmorrhage is seen, while at others the liver tissue seems more or less destroyed, leucocytes alone or mixed with red corpuscles abounding in the damaged tissue Plague bacilli occur in moderate clusters, being often situated at the periphery of the necrotic areas Similar but larger nodes are encountered in the spleen, *bacilli in groups* occurring near their circumference

Tarabagans suffering from such 'nodose' plague have been recorded in the past. Thus —

(α) A few animals, including that shot in the fields by Barykin in the year 1907(20), showed small greyish nodes in the spleen

(β) One naturally infected animal found by Sukneff in 1923 had some bulging nodes in the lungs, many nodules and hæmorrhagic spots in the liver, the spleen of this animal was partly eaten by eagles so that its nature could not be ascertained(19) Histologically the condition in the liver, though further advanced, was similar to that observed in Tb. 5-*b*(21)

(γ) One tarabagan infected conjunctivally in the course of our former winter experiments (1922 23) and succumbing on the 17th day after infection showed numerous pin head nodules of yellowish white colour in the liver and larger white nodes (size lentil) in the spleen

Cultures from the cases β and γ were somewhat impaired in virulence For this and other reasons we were inclined to consider such nodose changes as the result of a sub acute or even chronic stage of the disease As shown by our recent experience, such alterations may develop comparatively quickly, so that one must be chary of hasty conclusions We believe that in the tarabagan as in other rodents such forms as acute sub acute and chronic ought not to be separated by any sharp arbitrary line In all probability transitory forms between these also exist

(*d*) *Animals succumbing to plague after awakening from hibernation* —The two animals belonging to this group may now be described —

(i) Tb 34, infected on 3rd January 19-7 was up to 10th January kept in the outhouse later in the laboratory stable Slept well with short interruptions up to 5th March From then onwards it was mostly in a drowsy condition though eating a little food at times When stirred it did not bark, was not shy of human beings and generally remained listless When occasionally taken out of the cage it did not resist or attempt to run away sometimes the hind legs looked paralysed. On the whole it gave the impression of being affected by a chronic disease (plague?) rather than in a state of hibernation That the latter condition prevailed became evident early in (2nd) May when the animal began to react better displaying its teeth when approached but not barking A week later (9th May) it was wide awake greedily feeding when a carrot was thrown in This condition remained the same up to the morning of 12th May when it was last seen alive Next morning (13th May) it was found dead i e 130 days after infection

P M —Little fat though not emaciated

No marked local reaction Small but markedly congested bubo in left inguinal region some hæmorrhages in the fascia nearby Right inguinal glands slightly enlarged and congested cervical glands somewhat congested but not enlarged

Lungs œdematous right shows large areas of congestion

Numerous petechiæ on epicardium.

Liver not enlarged brownish yellow in colour some peri hepatitis in form of white linear thickening of the tissue

Spleen softer than normal though not enlarged congestion at places Retro peritoneal hæmorrhages

No other conspicuous changes

Bacteriological examination —Smears from bubo heart lung and spleen positive Cultures from bubo no growth from heart lung liver and spleen positive though somewhat contaminated Two guinea pigs infected immediately at post mortem succumbed to plague The first which was pricked with a needle dipped into the bubo died on the third day, while the second rubbed into the shaved skin with material from bubo heart and lung succumbed on the fourth

Histological examination —Sections from the bubo show as far as the gland tissue is concerned appearances similar to the acute cases Severe hæmorrhage is present leading at places to a disintegration of the structure Plague bacilli are very numerous often arranged in big clusters at the periphery of the gland The capsule however shows at places marked thickening and cell proliferation but leucocytic infiltration is absent The tissues near the bubo are much congested, hæmorrhages are occasionally met with but no leucocytic infiltration

A slightly enlarged *lymph gland from the right groin* shows much congestion but no hæmorrhage. Plague bacilli are numerous in the larger blood vessels, but scanty outside them The capsule is not *perceptibly changed* *The surrounding connective tissue is less congested than in the case of the bubo*, no leucocytic infiltration could be seen only small hæmorrhages

The *liver* shows at places thickening of the capsule Congestion and parenchymatous degeneration are present Fairly numerous plague bacilli are noted in both vessels and capillaries, sometimes in small clusters

The *spleen* is much congested The Malpighian bodies seem comparatively small, the trabeculæ prominent Plague bacilli occur in large numbers but are more evenly distributed than in the foregoing cases, so that no big clusters are met with

The *lungs* are congested and show foci of broncho pneumonia, the exudate is mostly cellular, red blood corpuscles being more numerous than white ones *B pestis* are plentiful, rarely in groups

The *kidneys* show congestion and parenchymatous degeneration, plague bacilli being noted within the vessels only.

In the *retroperitoneal tissue* one sees large hæmorrhages, here the bacilli are mostly grouped together in small clusters or loose nets

(*u*) Tb 4 *a* was infected on 17th January, 1927, and kept throughout in the outhouse Slept well with almost no interruption up to the beginning of April Being occasionally up from 4th April, the animal was wide awake on 11th April on this day it was seen to sit on the straw in its cage but to hide itself immediately when approached Was then well and feeding up to 13th April when seen for the last time before death Found dead on the morning of 15th April, i e, 88 days after infection

P M—Medium sized animal, still moderately fat

Some reaction is noted at the site of infection (superficial layer of musculature) where congestion and perhaps some infiltration are present Left inguinal glands slightly enlarged but not markedly *congested*

Lungs are œdematous, anterior parts pale, areas of congestion in dorsal parts of both lower lobes

Liver is congested and shows some indistinct sub capsular hæmorrhages Some peri hepatitis over left lobe in form of a net of white, thickened tissue

Spleen not markedly changed

Stomach shows petechiæ below mucosa, is full of bile stained liquid Duodenum and upper part of jejunum much congested their contents bloody Sub serous hæmorrhages on duodenum

Bacteriological examination—Smears from spleen show numerous bipolar stained, gram negative bacilli from lung somewhat atypical gram negative bacilli In films from bloody intestinal contents, bacilli similar to *B coli* besides larger gram postive bacilli

Cultures from heart, liver, spleen are typically positive, those from the lung somewhat atypical but suspicious Culture from intestine is negative for *B pestis*

Altogether five guinea pigs were inoculated One, receiving a dose of culture from the intestine into the shaved skin, survived, the others all succumbed to plague, thus

(*α*) Pricked with material from the site of infection, died on 3rd day,
(*β*) Rubbed „ „ „ lung and intestine, „ „ 7th „
(*γ*) „ „ liver *culture*, „ „ 7th „
(*ε*) „ „ lung „ „ „ 9th „

Histological examination—After prolonged search a small abscess is found at the *site of infection* The cells at the periphery stain fairly well, those in the centre have undergone necrotic changes, so that a diffusely stained mass is present showing at places indistinct nuclei The connective tissue near the abscess is markedly changed, rich in cells and blood vessels it has the aspect and arrangement of granulation tissue Small hæmorrhages are seen at places *but no agglomerations of leucocytes* No bacilli may be distinguished in the centre of the abscess, though they are fairly numerous at the

margin occ rr nr both n typical an l n olut on f rn s, the former occas all in cl st rs B l the abscess pl e bac ll are pl nt ful tl e vessels l ut s arce i the t s w tl tl x x p t f the hæmorrha areas even l re no large clusters of bact r a are see

One enlar ed *l t inju nal gland* shows u l r tl e n croscope sm e c nm t an l sn all l n or r areas The connect ve tiss e see ns n rcase l vol n at tl e cost of th p renel na The arran e ent of the ba ll rese nbles tl t n tl e co nect ve t s o of th st f fect Tl cap ule of the gl n l s th cken d w tl cell prol f rat o at pl s Some co gest ex sts aro l tl e gland l ut no n arked peri aden t s

The aps le of the *l er* is tl ckened cell prol f rat n is t n t ceabl mark l o gest n is present throu hout the organ tl occas o al l æ orrhages Tl e l ver cells sl ow s n s of f tty degenerat on Pla ue bac ll are plent ful ess l an l cap ll r s at a l ear tl e l æ or l ag s they also occur outs le the v ssels, now and tl en for ng small cl sters

The *spleen* is very r ch n bloo l Caps l s som wl at tl ck ned an l lo s s s of cell prol f ra t n Smalln ss of the Malp l an bod es an l pro n nc of the f ll l s nay l o not d l t tl s is not so consp cuo s as n the forego ng case Pla ue bac ll are very i un er s tlo l l p cl sters are absent

The *lungs* sl w gen ral con est on a l fo of bron l o pne n o a espe lly n ar the s rf c Here the al eol are f lle l w th a serou l ss often a cell l r exu late tl e latt r l bl od rp s cl s preva l Bac ll are n od rately n merous n the foc oft n sn all gro ps otl er p ts f the lun they are present the vess ls an l cap lla s nly

The *k lneys* are c n sted Pare l yma ous d gen rat on present Ba ll are q u e n vessels and cap l ar s

Sect ons from the *sto nach* how extens ve subm u o s ha n rrl age conta n ng l ac ll gro ps The all of the *s all intest ne* s n uch cong sted w th hæ o rl a es at pl es exte l to tl s f of the mucosa B clusters of bac lli o cur a l around the hæ orrl a es

It can thus be seen that two of our 14 tarabagans infecte l wl ile sle p ng contin ie l to l ibernate They succun bed to plague with l acterem a in tl spri g after they ha l been up and apparently well for a few days in one of these two the hibernation period appeared prolonged *

The bacteria present at the time of death in the l lo l a i l organs were in one animal (Tb 4 *a*) l ut little impaire l in virulence if at all in the other they may almost be sai l to have increase l in virulence

The question as to where the plague organisms are preserve l in s ch an n als until the disease becomes manifest is not yet fully estal lishe l There is little l ubt that in Tb 4 *a* they remained at the s te of infection In Tb 3 *a* l owever n local reaction could be letected an l though some older cl ange may l e pres nt in the inguinal glan ls we cannot aff rm if the bacilli reacl e l tl em at an early stage of the infection Some chron c lesions were noticed in liver an l spl en of both animals but it is d fficult to gauge their signif cance Possil ly they were caused by toxins circulating in the blood We hope to elucidate all these questions during the next winter

---

* It is hardly necessary to ment on tl at an acci lental nfect on of the an mals du g tl e observat on s o t of quest on O r ta al a ans kept at Harl n s n e s mmer 19 6 we e free fron para t s No experiments w th rats or th r flea-bear g an mals w re perf rm l thr ho t th whole t m The few nfected g ea p s the laboratory stal le w re placed n l uckets away from the tarabagans The latter were k pt n n l vidual cages and every poss ble preca tion was taken a a st any unlikely cont n ncy of fect on throu l f od an l the like

G Summary and Conclusions

Evaluating the results of our latest investigations we must admit that the two animals surviving up to spring had contracted infection *during and not before* the onset of hibernation while all the twelve others died during winter, six of acute and sub acute plague As stated above, conditions are certainly much more favourable in nature than under artificial laboratory conditions It seems therefore improbable that the percentage of rapidly evolving plague cases can be as high as we have witnessed in our experiments Also it is probable that in some of the naturally infected animals plague does not develop at all or remains localized, resulting in recovery Be this as it may, there is little doubt that the tarabagan fleas like those of the susliks, play an important part in spreading the disease in winter as well as in summer and are able to preserve the virus especially during the cold season for lengthy periods Supplementing the knowledge attained by our experiments with the above considerations, we can see how plague is propagated among the tarabagans from year to year

Summarizing our knowledge as obtained from the tarabagan together with that of wild rodents elsewhere, and comparing the results with facts established in regard to the domestic rats, we may establish the following conclusions —

(1) The occurrence of rodent plague with bacteremia is a *sine qua non* for the propagation of the disease in the wild as well as in the domestic species

(2) Cases with chronic plague (in the strict sense) therefore do not play any important role in the preservation of the virus

(3) Besides cases of acute and sub acute plague carriers with bacteremia may intervene, their significance is however, not yet fully established

(4) The hibernation period to which some of the wild rodents suffering from natural plague are subjected, is not a hindrance to the perpetuation of the disease, but on the contrary an *indispensable link for the preservation of both the virus and the species*

(5) In order not to confound the issues, no mention has been made in the text regarding migration However, this may be important in two ways

(*a*) *Immigration* of healthy animals into an infected locality

(*b*) *Emigration* of infected animals or carriers of infected fleas into a healthy region

It is evident that in the first instance an impetus would be given to any enzootic present, while in the second case ample fuel would be provided for the virus regardless of its fate at the place of origin

APPENDIX

Tabulation of Temperatures

(a) In the unheated outhouse (b) in the basement of the laboratory where the animals of the series (winter 1926 27) were kept

Date	Temperature in centigrade		Date	Temperature in centigrade	
	(a)	(b)		(a)	(b)
December 2	— 7		January 14	—12	
" 3	— [illegible]		17	—1[illegible]	
6	— 9		19	—16	
8	—10		21	—17	
9	—12		24	—17	
" 11	—12		26	—16	
13	—12		2[illegible]	—16 *Mean*	
15	—13		31	—15 —12·5	12
18	—13				
20	—12		February 3	—14	
22	—11		5	—13	11
" 24	—12·5		7	—13	12
" 26	—13		8		10·5
28	—14 *Mean*		9	—14	
30	—17 —*11·3*		10		11
			11	—13	
			14	—11	11
January 2	—10		18		10·5
3	— 9		19	—10	
4	— 8		21	— 9	
" 6	— 8		22	— 9	
8	— 6		24	—10	11
10	— 8		" 26	— 9 *Mean*	— *Mean*
" 12	— 9		28	—10 —11·25	10 +10·9

APPENDIX—*concld*

Date		Temperature in centigrade		Date		Temperature in centigrade	
		(a)	(b)			(a)	(b)
March	4	— 9	1°	April	8	° *Mean*	
	5		10		13	*8 + 4 0*	11 5
	7	— 8	13		19		13
	9	— 8	16		°0		*11*
	11	— 7			°1		15
	14	— 7	11		°		1°
	16	— 8	10		°3		14
	18	— 6	*11*		°5		1°
	19	— 6	—		°8		13 5
	°1	— 4			°9		15 *Mean*
	°3	— 3	11		30		15 +13 1
	°5	— 1	1°				
	°8	1 *Mean*	1° *Mean*	May	3		1°
	30	4 —4 8	+11 8		4		13
					5		*16*
April	1	4			9		14 *Mean*
	4	°	1° 5		11		14 +13 6

REFERENCES

(1) Summary on the Etiology and Epidemiology of Plague Calcutta 1908 pp 80 87

(°) *Jour Hyg* 1907 VII p 470

(3) WILLIAMS (19°6) *Mil Surgeon* 19 6 IIX p 1ω *Amer Jour Trop Med* 19°υ VI p 387

(4) MCCOY (1911) *U S Pub Health Bull* No 43

(5) CRANNELL and DIXON (1918) *Mthly Bull Calif State Comm Hortic* VII p 597

(6) HARRISON *Mthly Bull Calif State Dept Agriculture* 9 0 IX p 187

(7) MCCOY (1910) *New York Med Jour* October 1st

(8) RUCKER *Plague in Lower Animals*

(9) PIRIE The Plague Problem in South Africa Publ of the S Afr Inst f Med Res No XX 19°7

(10) NIKANOROFF (19°υ) *Vj Microbiol and Epidemiol* IV 3 p 34

(11) Stepanov (1924) *Rep Saratov Antiplague Conf* p 24 Chiski *Ibid*, p 85

(12) Koltzov (1917) *Vratchebnaya Gaz*, p 147

(13) Nikanoroff (1924) *Rep Saratov Antiplague Conf*, p 122

(14) Gaiski (1926) *Vj Microbiol and Epidemiol*, V p 3

(15) Golov and Joff (1925 26) *Ibid* IV 4 p 19 and V p 239

(16) Dujardin Beaumetz and Mosny (1912) *Compt Rend Acad Sci* CLV p 329

(17) Suknev (1924) Results of Invest of Transbaik Endem Area in 1923 Chita

(18) Wu Lien Teh Transactions of 6th Congress F E A T M, 1925, II p 815 and A Treatise on Pneumonic Plague Geneva 1926 and 1927

(19) *Idem* Transactions of 5th Congress F E A T M 1923 p 305

(20) Barykin (1909) Russki Vratch 1909 No 15 p 518

(21) Wu Lien Teh and Lin Chia Swee (1923 24) *North Manch Plague Prev Serv Rep* p 172

EXPLANATION OF PLATE III

(a) No 5

Tb 6-a Site of infection

Fig 1 Shows more diffuse leucocytic infiltration and hemorrhage much congestion

(b) No 8

Tb 6 a Site of infection, Kossel staining

Fig 2 Shows same changes as (a) and clusters and nets of *B pes*

(d) No 4

Tb 4-a, Site of infection

Fig 3 Shows an old abscess with necrotic changes in the centr

(f) No 7

Tb 7 b, Bubo

Fig 4 Shows marked congestion and hemorrhages at plac caseation

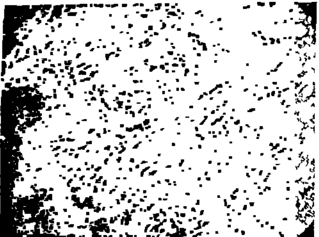


Fig 1

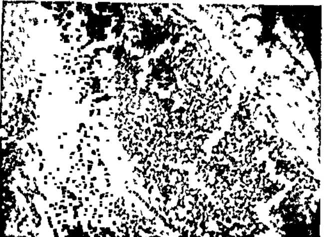


Fig 2





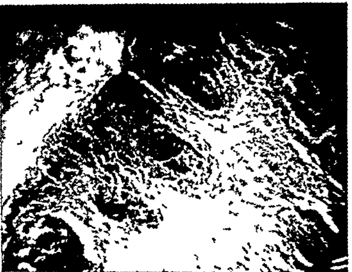
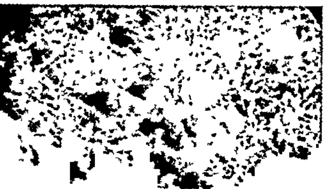


Fig 5



Fig 6





EXPLANATION OF PLATE IV

(g) No 11

Tb 3 b, Bubo

Fig 5 Shows, as far as the gland tissue is concerned appearances similar to the acute cases (severe hæmorrhage etc) Capsule shows at places marked thickening and cell proliferation, but no leucocytic infiltration

(h) *No* 14

Tb 3-*a* *Bubo*

Fig 6 Shows far gone caseation only remnants of the lymphatic tissue to be seen among necrotic masses

(i) *No* 13

Tb 5 *b* *Liver*, *Kossel staining*

Fig 7 Shows an area where the liver tissue is destroyed many leucocytes being present in the damaged tissue Plague bacilli are seen in moderate clusters, often situated at the periphery of the necrotic area

(j) *No* 12

Tb 5 *b*

Fig 8 Shows a similar no le in the spleen

STANDARDIZATION OF HAFFKINE'S PLAGUE PROPHYLACTIC

BY

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AND

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SINCE the discovery of the plague prophylactic in 1897, it has been observed that the different brews of the vaccine varied in their potency

In order to ensure that a fairly uniform and potent vaccine is issued from the laboratory for general use it seemed necessary that the prophylactic should be standardized

This paper deals with a method which we have employed since the beginning of 1926 every brew manufactured has been tested on rats for its potency

Before describing the actual technique employed for estimating the potency of the different brews of the vaccine the results obtained therefrom and the method we have adopted for standardizing the vaccine, it is well to draw attention to some important facts relating to Haffkine s plague prophylactic

Haffkine's plague prophylactic is essentially a sterilized broth culture of plague bacilli

For its manufacture the nutrient broth employed is an acid digest of goat's meat without the addition of commercial peptone, the organisms used for seeding the broth are originally obtained from human cases of septicæmic plague whose virulence is maintained by successive passages in Madras rats, the inoculated medium is incubated at room temperature (86°F) in the dark for a period of six to eight weeks it is then sterilized by heat at 60°C for 15 minutes, to ensure further its sterility it is carbolized to contain 0 5 per cent of the antiseptic

The prophylactic dose recommended for man is 4 c cs, but should the vaccine be employed within three months of its manufacture a dose of three c cs is recommended

Reaction of the nutrient broth —The reaction of the broth employed for the growth of plague bacilli is faintly acid and has been adjusted till lately with the aid of litmus paper

D'Aunoy s(1) studies on *B pestis* showed that optimum growth occurs at pH 6 2 to pH 7 0 or slightly on the acid side in veal infusion broth to which one per cent proteose peptone was added

Our(2) examination of 100 samples of the acid digest broth in terms of the hydrogen ion concentration and determined by the colorimetric method showed that the hydrogen ion concentration of the culture medium varied from pH 6 6 to pH 7 4 and that in 85 samples it lay between pH 6 6 and pH 7 0

Virulence of the bacillus employed for sowing the broth —Since the commencement of the manufacture of the prophylactic Haffkine(7) maintained that for the production of a potent vaccine it was necessary to employ highly virulent plague bacilli for sowing the broth , for this purpose he used whenever possible, a culture obtained directly from a plague patient or one whose virulence was maintained by frequent passages in rats

Bannerman(8) found that growth on agar for 23 days had the effect of lowering the virulence of a particular plague germ 250 times Our(3) experiments with vaccines prepared under identical conditions one from a five years old agar culture of *B pestis* isolated from a human case at the Maratha Plague Hospital and the other with the same culture whose virulence was raised by passage through a series of rats 41 times by the cutaneous method showed that the vaccine prepared from the virulent culture produced a higher degree of immunity in rats

Incubation for six to eight weeks —Haffkine(7) to accumulate for the prophylactic a large amount of toxins incubated the inoculated broth for five to six weeks at the end of which period he noticed that the bodies of the microbes became extremely deteriorated and the growth entirely stopped From this he inferred that the liquid in which the organisms grew was exhausted of its nutrient elements to support further growth Klein(10) also assumed that the growth of plague bacilli stopped because the nutritive material in the broth is exhausted

Bannerman(9) found that the inoculated broth when incubated for six to eight weeks became highly alkaline, and that a broth containing the equivalent of two per cent of normal alkali inhibited the growth altogether though it did not kill the plague germ Further he found that the production of alkali by plague bacilli in broth is regular, increasing from week to week to the maximum which is attained in about six weeks

Our(2 and 3) experiments on different brews of the prophylactic also showed that during the growth of plague bacilli in broth the medium becomes alkaline and this alkalinity steadily increases in amount from week to week reaching a maximum point between the fifth and the eleventh week of incubation , this is followed by a series of slight rises and falls in the amount of alkalinity throughout the period of incubation extending to 176 days Even though the broth culture is incubated for a period of 176 days the organisms are still viable and a good growth is obtained on agar slopes when subcultured The amount of alkalinity present in the vaccine has apparently no influence on its potency as estimated in rats

Stevenson and Kapadia(5) *found that vaccines incubated from six weeks to* two months were very much more potent than those incubated for more than three

months, it was therefore decided, in 1911, to despatch from the laboratory vaccine which had not been brewed for a longer period than eight weeks

Our(3) experiments on the effect of incubation period on the potency of the prophylactic showed that the potency increased with the period of incubation, that it reached its maximum at the end of five weeks, and that it remained constant up to thirteen weeks of incubation After this period further incubation resulted in the very gradual diminution of its potency

Variation in the potency of different brews of the prophylactic —As early as 1897 98 Haffkine(7) found that the brews of the prophylactic always differed in their protective effect in man He maintained that, apart from the biological factors which are little understood the medium employed for the growth of the organisms their virulence the period of incubation and the density of growth had an influence in the production of a potent vaccine Bannerman(8) also observed the lessened effect produced by different brews of the prophylactic, and attempted the production of uniformly potent brews by placing reliance on the exact procedures employed in obtaining fresh and virulent germs and for securing a nutrient broth of nearly constant composition

Since then medical officers engaged on plague inoculation campaigns have from time to time drawn attention to either excessive reactions following the inoculation of a particular brew or to the absence of any such reactions following the use of a particular brew

In our(3) study on the plague prophylactic we have estimated the potency of several brews manufactured for use and found that while the brews maintained a fairly uniform standard of protective value, yet some of the brews manufactured under identical conditions showed protective value considerably below the average

At the Lister Institute Schutze(11) tested the immunizing value of one of our brews namely No 427, in guinea pigs by a method different from ours and obtained a percentage (survivors) immunity of 14, while we with the same brew tested on rats obtained a percentage (survivors) immunity of 13 9 by a method which will be presently described These results are significant

Although the main question as to why the different brews of the prophylactic vary in their potency is still unanswered, our recent knowledge on the behaviour of the rough and the smooth colonies of *B typhosus* and their antigenic properties suggest *a useful line of enquiry in connection with the antigenic properties, and the* toxicity of the rough and the smooth colonies of *B pestis* and our laboratory experience of 1926 seems to suggest that the presence of a large number of organisms in the culture which develop the rough colonies has a deleterious effect on the potency of the individual brew

Immunizing dose of the prophylactic for man —The methods of finding out the immunizing properties of the plague prophylactic on man are not so simple as might appear at first sight the reason being that the crucial test of submitting the immunized subject to a dose of lethal virus is inadmissible in this case Haffkine(7) first studied the immediate physiological or pathological effect of the prophylactic

on himself with a dose of ten c cs , he then inoculated several hundred persons of all ages and sexes with a maximum dose of 2 5 c cs for an adult man and concluded that the effects produced in the inoculated did not markedly differ from the effect of the cholera inoculation and that the prophylactic inoculation did not indicate the possibility of any evil consequences to the general health

This was the strongest material he ever had as he could never produce a material which, when injected in smaller quantities than this, would produce an amount of fever which he had agreed conventionally to consider as an indication of a sufficient reaction and of a sufficient protection conferred on the inoculated The indication which he had adopted consisted in a rise of temperature reaching 102°F on the average He therefore called the dose of 2 5 c cs the standard dose It was, however, only in exceptional instances that he had a material of the standard strength Having been prepared from the beginning for the fact that the brews turned out would vary in their quality, and wanting to have the result of inoculation fixed, he had to vary the dose accordingly

In operations at Kirkee and in Damaun those inoculated with weaker material which produced a smaller amount of reaction showed afterwards a higher mortality, and people who were inoculated with a larger dose of the weaker material gave a lower mortality

When plague broke out in His Majesty's House of Correction at Byculla, Bombay, in January 1897, the option of inoculation was offered to the prisoners and a dose of three c cs was used , the injection of three c cs of the prophylactic(7) seemed to be sufficient to effect the desired protection, no repetition of inoculation being necessary to arrest an outbreak

In 1905, Bannerman(8), from the results of animal experiments fixed the minimum dose of the anti plague vaccine at an average of five c cs and considered it safe to administer this dose even to those living in infected houses

In 1911, Liston(1) advised the dose to be reduced from four c cs to three c cs to avoid the unpleasant symptoms produced by fresh vaccine if used within three months of its manufacture In doing so he wrote that 'the protection afforded by the reduced dose was probably less than that produced by the larger dose, but we had to bear in mind the fact that a severe reaction following inoculation always tends to make the operation unpopular '

Standardization of the Plague Prophylactic

In 1905 Bannerman(8) attempted the standardization of the prophylactic and found the methods of standardizing different brews by (*a*) the febrile reaction produced, (*b*) the opacity test, and (*c*) the amount of alkali present in the vaccine, were all wanting

During the same year, Liston(8), by a modification of Wright s method of counting the number of germs in a given culture fixed as a suitable standard 'the maximum quantity of a vaccine which will, in 48 hours, protect a guinea pig weighing 250 to 300 grammes against at least 100 times the lethal dose of a plague culture

isolated from the body of an animal not more than one week previously' Until the beginning of 1926 the standardization of the prophylactic consisted in the exact procedures employed during its manufacture Since then every brew manufactured in the laboratory has been tested on rats for its potency and such of the brews which yielded a low degree of potency were re tested before the vaccine was rejected By this method it is hoped that a fairly uniform and potent vaccine could be issued for general use

The usual methods of standardizing vaccines, namely, the counting method the opacity method and the gravimetric method are inadmissible in the case of the prophylactic because the culture medium during the long period of incubation, becomes highly alkaline resulting in the lysis of most of the organisms, and we(3) have already shown that the immunizing agent in the finished product is the clear supernatant fluid and not the sediment

Technique for estimating potency of the prophylactic —We shall here very briefly describe the technique employed for estimating the potency of the prophylactic, as we have already discussed at some length the considerations which led us to adopt this method in our paper namely Notes on the potency of Haffkine's plague prophylactic published in the *Indian Journal of Medical Research* Vol XIII No 4 April 1926

(1) *Experimental animals* Rats (*Mus rattus*) imported from Madras have been employed as test animals

TABLE I

Susceptibility of Madras rats to experimental infection with plague

Years	Rats used	Total deaths in 15 days following on infection	Percentage mortality
1923—1925	482	450	93 3
1926	360	336	93 4
1927 to the end of October	210	198	94 3
Total results	1 052	984	93 6

The above table indicates the uniform susceptibility of Madras rats to experimental infection with our test dose of plague

In the course of our study, we have also estimated the susceptibility of rats (*Mus rattus*) caught in the city of Bombay to experimental plague infection using the same test dose as above

TABLE II

Susceptibility of Bombay rats to experimental infection with plague

Years	Months	Rats used	Total deaths in 15 days following on infection	Percentage mortality
1924	June	100	45	45 0
1925	May	200	130	35 0
1926	April	110	59	53 7
	May	50	13	26 0
	June	50	8	16 0
	July	50	4	8 0
	August	200	12	6 0
	September	150	32	21 4
	October	120	43	35 9
	November	120	25	21 0
	December	120	25	21 0
1927	January	120	25	21 0
	February	120	24	20 0
	March	120	33	27 5
	April	100	20	20 0
	May	120	26	21 6
	June	120	21	17 5
	July	120	17	14 1
	August	120	12	10 0
	September	120	13	10·8
	October	120	34	28 3
Total results		2 450	621	25 3

The above table not only indicates the high degree of immunity enjoyed by Bombay rats to experimental infection, but also shows that this resistance to infection is considerably higher during some months in the year

(2) *Immunizing dose of the prophylactic for rats* —Stevenson and Kapadia(4), in their experiments to determine the influence of the length of incubation period on the efficacy of the prophylactic, found that (*a*) the amount of immunity produced

in rats was to some extent dependent on the dosage of the vaccine employed, (*b*) a dose of 0 25 to 0 5 c c was sometimes sufficient to immunize more than 50 per cent of rats against subcutaneous infections of virulent plague, and (*c*) 0 025 c c formed a good immunizing dose for rats as it did not produce a high death rate from toxæmia De Smidt(12) in his commentary on 'The Plague Problem in South Africa,' considered that his conclusion namely, the correct effective dose for rats is from $\frac{1}{16}$ to $\frac{1}{8}$ the safe effective dose for humans was strikingly supported by the results of his analyses of experiments carried on rats with different anti plague vaccines We have employed throughout 0 5 c c of the prophylactic for immunizing rats weighing from 65 to 80 grammes, as we have attempted in these experiments not only to estimate the potency of the different brews of the prophylactic but also their toxicity

(3) *Interval between inoculation and infection of rats* —Haffkine(7) in human subjects Stevenson and Kapadia(5) in rats using the prophylactic and Rowland(4) using plague nucleoprotein for inoculation have shown that the immunity following the inoculation was very rapid beginning to manifest itself within 24 hours of inoculation Stevenson and Kapadia further showed that the production of immunity among rats increases in amount till the second or third day after anti plague vaccination and that 14 days after inoculation a considerable degree of immunity is still present In view of these considerations we have allowed an interval of seven days between prophylactic inoculation and infection with plague

(4) *Test virus for rats* —For estimating the potency of anti plague vaccines cultures of plague bacilli either grown on agar or in broth, and the spleen of a plague infected rat have been employed as the test virus To ensure the virulence of the test virus we have in our experiments used the spleen of a rat that had died of acute plague

(5) *Test dose for rats* —The Plague Commission in India(13) using the spleen of a plague infected rat varied the doses from 0 2 to 0 01 milligram for rats Later, Stevenson and Kapadia(5) also used the spleen of a plague infected rat as the test virus for estimating the potency of the prophylactic in rats In their first experiment they employed a dose of 0 0031 milligram but for reasons which are not obvious they varied the dose from 0 007 to 0 0019 milligram in their subsequent experiments

We have uniformly employed a dose of 0 003 milligram of the spleen of plague infected rat With this dose administered subcutaneously we obtained a fairly constant rate of mortality which is indicated in Table I

For the preparation of the test dose, we have employed the spleen obtained from a rat experimentally infected with plague by the cutaneous method We have here compared the daily mortality in rats following the cutaneous method of infection with that following the subcutaneous injection of 0 003 milligram of plague spleen

From Table III it will be seen that the percentage mortality by both the methods is about the same, and that the daily mortality following on the

TABLE III

Daily mortality among rats experimentally infected with plague spleen of a rat

Year	Rats used	Daily mortality from plague infection															Total deaths in 15 days following on infection	Percentage mortality
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
A Cutaneously infected																		
1926	550	11	33	271	145	37	12	7	1	0	2	0	1	1	0	0	526	95 6
1927*	436	7	16	178	146	42	13	2	3	0	0	0	0	0	0	0	406	93 1
Total results	986	18	53	449	291	79	25	9	4	0	2	0	1	1	0	0	932	94 5
B Subcutaneously infected with 0 003 mg plague spleen																		
1926	580	4	33	210	165	63	31	18	6	5	5	2	1	1	2	0	546	94 1
1927*	340	0	0	118	115	41	27	7	4	3	1	0	3	1	1	0	321	94 4
Total results	920	4	33	328	280	104	58	25	10	8	6	2	4	2	3	0	867	94 2

* Up to the end of October

subcutaneous method of infection with the test dose tends to spread over a slightly longer period

(6) *Estimation of potency of the prophylactic in rats*.—The Plague Commission in India(13) based the index of immunity on the number of deaths following on infection up to 22 days in considering the results they excluded all deaths occurring on the day of infection and the day following for they regarded them as due to handling and not to plague

Stevenson and Kapadia(5) based their index of immunity on the number of survivors from the 8th to the 13th day after infection, in considering the results they counted as having died of plague only those animals which showed plague bacilli in smears made from either their organs or buboes

We have estimated the potency by the number of survivors at the end of 15 days after infection Out of the 2,052 rats infected by the Plague Commission in India, only eight died of plague between the 16th and the 22nd day after infection, that is, 0.003 per cent In our experiments of the rats that had died between the 10th and the 15th day after infection, we found that a small number had died of acute plague We have stated the results by including all deaths which had occurred from the time of infection without any omission or correction and expressed them as total deaths following on infection in 15 days, although every experimental rat that had died has been studied by post mortem and microscopical examinations

(7) *Results*.—*We have herein tabulated the results obtained with the brews of* the prophylactic, examined by the above technique, during the years 1926 and 1927 until the end of October

TABLE IV

Variation in the potency of different brews of the prophylactic

Brew	Percentage Immunity (Survivors)	Brew	Percentage Immunity (Survivors)	Brew	Percentage Immunity (Survivors)	Brew	Percentage Immunity (Survivors)
For the year 1926							
444	15.4	451	18.9	458	44.8	465	26.9
445	44.0	452	19.6	459	36.3	466	20.0
446	40.9	453	36.5	460	45.0	467	36.0
447	52.9	454	48.0	461	17.3	468	42.8
448	44.0	455	41.6	462	35.0	469	49.0
449	46.0	456	31.0	463	31.5	470	28.8
450	36.7	457	40.7	464	37.2	471	22.6

TABLE IV—*concld*

Brew	Percentage Immunity (Survivors)	Brew	Percentage Immunity (Survivors)	Brew	Percentage Immunity (Survivors)	Brew	Percentage Immunity (Survivors)
			For the year 1926—concld				
472	18 0	481	22 0	491	27 2	33	34 6
473	30 1	482	25 0	492	34 6	40	30 0
474	48 1	484	17 2	493	25 0	41	27 5
475	21 4	485	20 0	24	29 6	43	44 4
476	25 0	486	30 7	26	34 4	44	29 1
477	25 4	487	17 3	27	18 5	45	31 0
478	18 8	488	50 0	28	25 9	49	40 1
479	23 5	489	18 5	30	33 3	50	28 5
480	17 8	490	33 3	32	28 8		
			For the year 1927 *to the end of October*				
51	23 0	74	41 1	95	34 4	123	30 0
52	43 0	76	47 6	96	44 4	124	36 6
55	40 0	77	37 0	101	49 0	126	43 8
57	32 0	78	33 3	102	41 4	128	31 0
58	31 2	79	52 3	103	51 8	130	20 2
60	52 3	83	28 3	104	46 4	133	43 3
61	20 0	84	47 6	108	31 0	136	40 3
62	27 2	85	38 2	110	39 6	140	26 6
65	40 0	89	39 1	112	28 0	142	26 6
71	64 0	94	38 0	120	42 8	144	25 8

From the above table it will be seen that during the year 1926, 63 brews have been employed to seed the culture flasks which varied from 120 to 260 flasks for each brew, and each flask of manufactured prophylactic provides on an average 250 adult human doses of 4 c cs Of these 63 brews no less than 29 brews had an immunizing value of less than 30 per cent for rats and of these 29 brews ten of them had a potency less than 20 per cent During the first ten months of this year 20 brews have been employed to seed the culture flasks, of these 20 brews, nine brews had an immunizing value below 30 per cent and none below 20 per cent

STANDARDIZATION OF THE PROPHYLACTIC

In order to ensure the supply of a fairly potent vaccine for human protectio
it seems necessary in the face of these variations to fix upon a suitable standard
potency for the prophylactic With this end in view, we have tabulated below t
average percentage immunity obtained with different brews of the vaccine examin
since 1923, together with results obtained among the human population(6)

TABLE V

Results of human inoculation with Haffkine's plague prophylactic

	Attacks	Deaths	Percentage Immunity (Survivors)	Years
Uninoculated	56 210	48 080	14 51	1902—1916
Inoculated	3 164	1 209	61 7	

Results of rat inoculation with Haffkine's plague prophylactic

	Infected	Deaths	Percentage Immunity (Survivors)	Years
Uninoculated	482	450	6 7	1923—1925
Inoculated	778	515	33 9	
Uninoculated	360	336	6 6	1926
Inoculated	2 895	2,030	29 8	
Uninoculated	210	198	5 7	1927 *
Inoculated	1,520	968	36 3	
Uninoculated	1,052	984	6 4	Total results 1923—1927 *
Inoculated	5,193	3,513	32 3	

* To the end of October

From the above table it will be seen that (*a*) the percentage immunity figure
among the uninoculated and the inoculated of both the human and rat populatior
bear a constant ratio of 1 to 5 (*b*) the percentage immunity figures for the rat popula
tion and for the human population are in the proportion of 1 to 2, and (*c*) the
average percentage immunity figure for rats is 32 3, which varies only slightly fron

those recorded for the three different groups of experiments Therefore, it seems possible by the technique we have employed to estimate the potency of the different brews of the prophylactic and to fix on an average minimum standard of potency for the prophylactic at 30 per cent immunity (survivors) for rats

Summary

(1) Rats imported from Madras for these experiments showed uniform susceptibility to experimental infection with plague the average mortality within 15 days of infection being 93 6 per cent

(2) Rats caught in the city of Bombay showed a high degree of immunity to experimental infection with plague the average mortality within 15 days of infection being 25 3 per cent This resistance to infection was higher during some months in the year

(3) The spleen of a rat which had died of acute plague is considered satisfactory for estimating the potency of an anti plague vaccine the dose being 0 003 milligram

(4) The percentage mortality in 15 days among Madras rats whether infected by the cutaneous method or by the subcutaneous injection of 0 003 milligram of spleen of a plague-infected rat was about the same namely 91 0 per cent

(5) The immunizing dose of 0 5 c c of the prophylactic for rats conforms to the considered effective dose for rats namely from $\frac{1}{16}$ to $\frac{1}{8}$ the safe effective dose for humans as 4 c cs is the dose recommended for man

(6) The method of estimating the potency of the prophylactic by the total deaths following on infection in 15 days is both simple and satisfactory

(7) Estimation of the potency of different brews by animal test showed that different brews varied in their potency and some were of considerably low protective value

(8) It seemed possible to standardize the potency of the prophylactic by animal test so that a fairly uniform and potent vaccine could be issued for general use

References

(1) D Aunoy — *Jour Inf Dis* XXXIII pp 391 and 396

(2) Naidu and Jung (1927) — *Ind Jour Med Res* XV 1 pp 135—139 XV 2 pp 335—341

(3) Naidu Malone and Avari (1926) — *Ibid* XIII 4 pp 823—834

(4) Stevenson and Liston (1911) — Report, Bombay Bacteriological Laboratory pp 3 and 23

(5) Stevenson and Kapadia (1924 25) — *Ibid* XII 1 pp 199—211 and XII 3 pp 553—559

(6) Morison Naidu and Avari (1924) — *Ibid* 2 pp 313—320

(7) Haffkine (1898 99) — Minutes of Evidence taken by the Indian Plague Commission Vol I pp 4 5 paras 32 34 36 p 6 para 47 pp 8 10 para 72 pp 27 28 65 Vol III pp 26451—26453

(8) BANNERMAN and LISTON (1905) Report, Plague Research Laboratory, pp 3—7
(9) *Idem* *Sci Mem Officers of the Med and San Depts of Govt* (*Ind* New Series No 33 pp 4 and 7
(10) KLEIN (1901 02) Thirty first Annual Report, Local Government Board
(11) SCHUTZE (1925) *Brit Jour Exper Path* VI p 208
(12) DE SMIDT The Plague Problem in South Africa *Kenya and La African Med Jour* IV, 7, p 218
(13) PLAGUE COMMISSION IN INDIA (1912) *Jour Hyg*, 12, December, Supplement p 231—254

DISCUSSION

The Epidemiology of Bubonic Plague

Dr E P Hicks (Shanghai) Col Mackie referred this morning to the importanc of recognizing the different species of *Xenopsylla* Dr Goyle's paper reiterates th importance of this I should like to emphasize another point from my observations i Shanghai Though there is not much plague there—in recent years only occasiona outbreaks—we have plenty of *X cheopis* The season for this flea is from August t January and this is the plague season in April and May there are practically none When plague disappears from Shanghai as it does for several years at a time, it must b introduced from outside The ports with which Shanghai is most clearly connected and in which plague is endemic are those of South China In these the disease flourishe in April and May when there are practically no *cheopis* fleas in Shanghai I think therefore Shanghai is protected by the difference between its *cheopis* season and th plague season of the ports in which plague is endemic The public health importanc of this is obvious If plague occurs in a neighbouring port while there are no *X cheopi* in Shanghai (i e April and May) there is no need to be unduly alarmed in Shanghai But if such an outbreak occurs during the time when *X cheopis* flourishes in Shangha (August to December) then full anti plague quarantine measures should be put int operation This applies to other ports which are in the same position

Dr L Fabian Hirst (Ceylon) May I offer my congratulations to Dr A N Goyl on his important contribution to the parasitology of plague I have been intereste in this question since 1913 when I first put forward the hypothesis that *X astia* might l a relatively inefficient vector of plague as compared with *X cheopis* I have subsequently reported the results of transmission experiments carried out in Colombo by a variety of different methods in 1915 and 1922—1924 mostly during the plague season Dr Goyle s results are confirmatory as far as they go, of my conclusions that *X astia* is a relatively inefficient vector of plague under tropical conditions They are particularly interesting in that they enable us to form a rough idea of the relative degree of drying power of the air required to check the *astia* and *cheopis* borne epizootic in experimenta cases of the orthodox type in what I call the inland plain type of station, i e, Lucknow city where the monthly variations in air temperature and air humidity are considerable It would appear that *X astia* tends to fall out of transmission at an earlier point in the saturation deficiency curve than *X cheopis*

I am glad to learn that Dr Goyle proposes to continue his experiments, and I hope that he will utilize the technique I have devised and described for studying the plague transmitting power of individual rat fleas of different species infected on the same host

at the same time By this means the actual cause of the difference in plague transmitting power of *X astia* and *X cheopis* will probably be revealed My own experiments suggest that the difference may be due to one of these factors, (a) a definite lethal action of *B pestis* on heavily infected *astia* analogous to that known to obtain in the case of bugs and lice it should be remembered that plague is a disease of fleas as well as rats and human beings, (b) a relative weakness of the *X astia* proventricular valve, and (c) a lesser longevity of the infected *X astia* when separated from its host

Comparative experiments with any species of flea must be so conducted as to bring out any essential specific distinction in habit, resistance to plague infection, viability when separated from the host or structure of the suctorial apparatus likely to be of importance in a state of nature I have produced evidence tending to show that X *astia* has a less unfed longevity than X *cheopis* If so infected X *cheopis* could be transferred a long distance and would be able to disperse the infection over a wider range under its own locomotive power than X *astia* under parallel climatic conditions These latter points would not be brought out by the experimental technique employed by Dr Goyle

In conclusion I should like to draw attention to a reference to my work in the papers by Col Mackie which is not strictly accurate Col Mackie states if I understand him aright that I never obtained successful transmission in the blocked *astia*—this is incorrect

I refer those interested to my memoirs on Researches on the Parasitology of Plague (page 198)

Dr F l Herelle (Egypt) Described an outbreak amongst Bedouins in Egypt in which the first cases were probably attributable to fleas received in a bag of grain but that the continuance of a small epidemic was probably due to human fleas no rodents being found in the desert area

Mr V D Pillai (Hyderabad Deccan) In 1912 at the All India Sanitary Conference Madras Capt W C Ross said —

'It seems that the most important differences in the habitations are that houses in England are of masonry and are largely rat proof and the drains are not open highways for the passage of rats but are closed and trapped so that the rats and men are kept separate' In Hyderabad a certain area was rebuilt and for years plague never had any effect on that area But in other parts and particularly in Secunderabad, every year plague has played havoc for the last 16 years There is no use in putting a system of underground drainage here Undeveloped and ill built areas in lanes require a different system The houses must have rat proof floors as also roofs The trap is useless for houses which are badly built and in Hyderabad we have at least 75 per cent of houses badly built The rubbish bin and house gutters where a very large quantity of food refuse is thrown makes the rat live there and wait for its food No refuse food should be thrown either in the dust-bin which remains unmoved for days or into the house drain, where the rat always remains for the food These are the unhealthy places and the attention of sanitary engineers should be directed towards the solving of this great problem in Indian towns

Lt Col C L Dunn I M S (United Provinces) I do not think that any of the recent discoveries on the epidemiology of bubonic plague, with regard to the more

efficient vectors, have produced anything practical to aid the administrative public health official to deal with epidemics more efficiently and less expensively than by de ratting Dr Goyle has indicated, in the latter part of his paper, the influence of humidity on the epidemiology of plague and he agrees with me that there are some grounds for believing that the villages in which plague persists through the dry hot weather in the United Provinces are villages with a high soil humidity which we call 'wet' villages This matter is being further investigated and we believe it may explain the peculiar distribution of endemic foci in districts with varying climates It is well known that in one village plague may break out year after year while in a village a few miles away it remains uniformly absent If these scattered endemic foci prove to be 'wet' villages we will have an epidemiological fact which will be of great use in the prevention of plague epidemics and in the extirpation of these foci from which epidemics begin every autumn

'Carry through' villages are very few in number so that it would not be difficult to take measures by drainage to render the soil unsuitable for the resistance of the infection through the hot weather

Col W H C Forster, I M S (Burma) I shall confine myself to an aspect of this question which was emphasized by Col Mackie, which is of special interest to those who, like myself are concerned chiefly with the practical application of the results of research and who moreover, have to deal mainly with a rural population I refer to the problem of recrudescence and in making these remarks I may say that they are based on an administrative experience covering 5 million cases of plague and a mortality from plague alone of 30 per *mille—a dismal experience which I sincerely trust is* unrivalled

The seasonal plague mortality curve of the Punjab shows a maximum in April declining to a minimum in August where for weeks at a stretch the province may be entirely free from plague From August the curve slopes up very slowly until December after which the rise increases rapidly Observations in 26 years have shown that the autumn mortality is almost entirely due directly or indirectly to recrudescence in villages in which the epidemic was incomplete on the onset of the hot weather in June Observations as to the rate of recrudescence in such villages so far collected show that it is approximately 30 per cent

The actual mortality in these villages is seldom considerable but the villages within a radius of five miles quickly become infected, supply the bulk of the mortality, and function as disseminating foci of infection

In 1926 a determined effort was made to prevent recrudescence in *the potential* centres by systematic de ratting The Ambala Division was selected for the experiment, a very large staff was employed and a complete list of all villages with an 'incomplete' epidemic, or in which the infection was so late that an incomplete epidemic could be assumed was prepared The list totalled 918 villages The original intention was to commence in August and have all listed villages de ratted three times by the end of October—experiments having shown that the villages could only be de ratted once a month Unfortunately floods and other difficulties prevented us from carrying out the original programme and so operations were continued till the 31st of December and the result then appraised Without discussing the methods employed I will give you the

result On the 31st December the recrudescence rate in these 918 villages was as follows

Villages de ratted	once	10	per cent
,,	twice	2 5	,,
,,	thrice	0 7	,,

Gross recrudescence rate 3 3 per cent as against an expected rate of 30 per cent In the case of 11 villages de ratted thrice before the end of October the recrudescence rate was nil In this connection, without claiming that the preventive effort was responsible for the entire result, I may say that the plague mortality in 1927 up to the time I left the Punjab was very close to the lowest on record

As regards the method by which the carry over is accomplished I am of opinion that when the matter has been fully investigated it will be found that the flea and not the rat is responsible In this connection I might add that when the late Major G Lamb I M S, and myself were working at this subject 23 years ago the former had collected evidence tending to show that infected fleas might survive for as long as three months

Dr B P Nigam (United Provinces) The modern conception regarding the term chronic plague is rather different from what it used to be a few years ago The pathological appearances described as chronic plague are merely stages in the process of recovery from the acute disease

After examining 3 000 rats at Azamgarh U P an endemic area of plague, I can bring forward the following results regarding chronic lesions —

Total rats examined	Macroscopic Lesions									Microscopic result showing *B pestis*
	Gland lesions				Spleen					
	Mesenteric	Axillary	Inguinal	Sub maxillary	Scar	Bisection	Necrotic foci	Abscess	Notched	
3 000		5	13	14	47	18	7	2	45	2 liver smears 3 heart smears 2 spleen smears

Percentage of chronic lesions	macroscopic	5 03
,,	microscopic results	0·23

The inoculation of guinea pigs with the spleen of the rat which showed *B pestis* in its smear, caused death in 36 hours showing *B pestis* in heart spleen and liver smears What relation this chronic plague is supposed to have with an acute epizootic is a problem with many writers, but none so far has reached any definite conclusion The possible

ways in which the disease may pass from one season to the other, in my opinion, may be as follows —

(1) The chronic lesions may light up into an acute condition, such as the bursting of an abscess into the peritoneum or a vein This view does not hold ground when we look to the practical side It has been noticed that adhesions seem to form readily so as to limit the lesion and most of the abscesses have thick fibrous walls, thereby lessening the chance of bursting

(2) Rats become infected with acute plague by eating those with chronic plague I have little reason to believe this either as by doing so the rats would show mesenteric buboes, none of which I have found I have observed in our laboratory at Lucknow that the rats often eat their own young, but these are generally the weak and young ones which again are little liable to be suffering from chronic plague having been born in the quiescent period

(3) It may be possible that the carriers among the rats may be constantly passing plague germs in their excreta, thus contaminating their food and soil and making the surroundings favourable for the disease We have not yet investigated this fact Cultural reactions from the soil may give good *results.*

(4) Again the fleas may be responsible, as Col Forster has said, but, according to Robertson, the rat flea survives the cold weather only in the immature form and there is therefore no transference of infection during the winter Even if introduced into a cold climate during the warmer months, plague infection would disappear with the disappearance of the adult fleas

In conclusion there is no direct evidence that chronic plague possesses any significance in the seasonal recurrence amongst rats of the infection in an acute form, nor is any evidence available which excludes this possibility

Lieut Col J Taylor, I M S (Burma) Neither the epidemiological or experimental evidence at present available is sufficient to enable us to come to definite conclusions as to the relative importance of *X cheopis* and *X astia* in the transmission of bubonic plague All workers in India have been successful in transmitting with both fleas, but the balance of evidence suggests that *cheopis* is the more effective vector At the same time the differences between these fleas under different climatic conditions are not settled, nor are the factors which may result in difference in transmitting power fully known The cage type of experiments are unsatisfactory as an indication of what will happen under natural conditions, and experiments in godowns with established rat and flea populations are necessary to ascertain the true facts These should be carried out in areas with different climatic conditions In the United Provinces there appears to be no relationship of plague to the relative distribution of *astia* and *cheopis* *Cheopis in India has a limited distribution with certain climatic relationships*, while *astia* has an almost universal distribution in the plains of an irregular character

Astia the flea of Madras city, transmits plague to Indian rats in Bombay, while Madras city is free from epizootic plague a climatic factor which appears to be of the greatest importance and of which it is necessary to determine the exact action

Referring to the recrudescence of plague discussed by Col Forster, I M S, the line of following up the villages infected late in the plague season, just before the onset of

the hot weather, for rat destruction later, to prevent the spread to neighbouring villages in the succeeding season, had been a matter of investigation by Lieut Col Khunhardt in the Deccan and it appears that the results now obtained in the Punjab form a very promising line of effective preventive work

Dr Charles W Young (China) In Li Hsien, in western Shansi, China, there is almost yearly an outbreak of plague which is at first bubonic, with deaths This begins in September As the weather becomes colder, the type of the disease changes into the pneumonic form After a few cases (always fatal) the epidemic dies out This sequence is repeated almost, or quite, yearly, but the disease does not spread outside this isolated mountain valley

Col Froilano de Mello (Portuguese India) Ayant fait la plupart de sa carrière dans l Inde, il connaît bien l'epidemiologie de la peste aux Indes Lorsqu il fut appelé pour Angola, il remarqua que la peste etait endemique à Loanda Ayant fait des recherches, vit que c'etaient des rats sauvages du genre Microtus et le Rattus Concha qui entretenaient la peste sous forme liminaire dans l interieur Et meme quelques noirs presenterent une épidemie de *furoncles* qui n'étaient d'autres que de vrais bubons pesteux En ce qui concerne l Inde, il faudrait etudier jusqu a quel point existe cette peste liminaire chez les rats et les musaraignes (Crocidura) comme test de l endémie pesteuse

Dr P T Patel (Bombay) In Bombay and the Bombay Presidency the rodent concerned is the house rat (*Mus rattus*) and sometimes squirrels are noticed dying during epidemics Many of the workers and also the villagers rely on the increased mortality as the indication of the coming of an epidemic of plague

THE PLAGUE PROBLEM IN THE SOUTH-EAST OF RUSSIA

BY

PROF S NIKANOROFF,

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As seen from the chart (Fig 1) plague is endemic in Transbaikalia, Turkestan and the South East of Russia The latter plague area is the most permanent, with outbreaks of annual occurrence and is of the greatest interest and importance

From the south and south east it is bounded by the Caspian Sea, on the west by the river Don, on the east by the Ural ridge and on the north by Latitude 50° N

How powerful plague is in this area is shown by the following diagram in which are given absolute figures of the number of plague cases in the South-East for the period from the year 1899 almost to 1927 inclusive (Fig 1)

As seen the interest of this area lies not in the absolute figures—they are not very great—but in the persistency of the endemic and in the location of this plague focus, in the European part of Russia and in the direct vicinity of the cultured world

There is nothing to wonder at in the fact that this plague focus is the subject of constant care by the special anti plague organization, the scheme of which is being referred to The efforts of this organization have succeeded in considerably elucidating the mystery which lately enveloped plague outbreaks, it has found out the cause of plague endemicity and the mechanism of its spread to man, and it has collected data sufficient for the organization of a rational plague control

It has been observed that plague outbreaks in the South East are periodical This periodicity which has become a law, is associated with *time* and *place* In this diagram are seen two sharp rises, one corresponding to spring summer, the other, to autumn winter (Fig 2)

The location of the outbreaks being duly considered, it will be clearly under stood that there are *two* distinct foci in the South East each with a different epidemiology In the steppe rather populated areas, with hard clay ground, susliks (*Citellus musicus* Men *C mugosaricus* Licht, *C. rufescens* Keis) are the

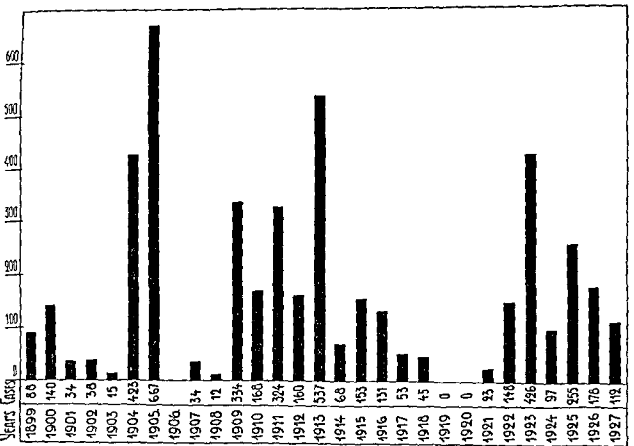


Fig 1 Absolute Figures of the Number of Plague Cases in the South East for the Period 1899 to 1927

principal carriers of plague These hibernating rodents stay in the open from *April till August* Plague epizoötics spread among them in May June Plague

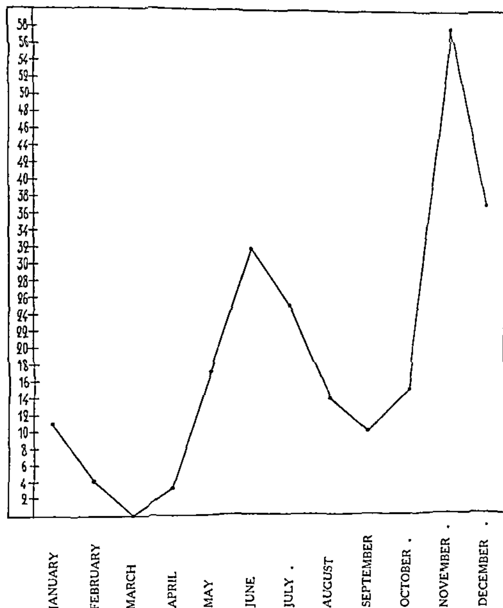


Fig 2 Periodicity of Plague Outbreaks for the Period 1899 to 1927

outbreaks in men closely correspond to these With the onset of hibernation of susliks, every possibility of primary infection for man disappears till the next

season The observations over the suslik area in spring summer have led to the conclusion that plague epizootics are of annual occurrence in this area The plague virus is carried over from one season to another

Attempts to discover the cause of this fact resulted in the observation that old rodents, which have hibernated seldom succumb to the epidemic, which usually assumes a mass character when the young become independent

Experiments show that the susceptibility of susliks to plague, rather high in summer subsides about the moment of hibernation and the disease runs a slow chronic course Experimental plague in susliks is obtained of 226 days' duration The slow course and local character of the plague in the susliks during hibernation has also been proved experimentally

It is highly probable that susliks can hibernate in the infected state, and, having awakened in spring and developed acute plague are able to transmit the infection to young susliks which are very susceptible to plague

The intermediate role of ectoparasites—fleas—is not excepted In this way plague, having once spread among the susliks is apt to be kept alive for long in their bodies the susliks being the reservoir of the plague the insects the transmitters We have therefore no reason to expect *self liquidation* of plague in the suslik populated areas

Much more complex is the mechanism of plague endemics in the second plague centre sandy areas The principal carriers of plague here are the house mouse and the sand mouse *Mus musculus* Linn and *Gebrillus meridianus* Buchan There are no rats at all in these areas I draw particular attention to this fact that the house mouse acts as a carrier and transmitter of plague here because recently Dr Jonge has said that the house mouse is innocent everywhere Long observations on rodents in this area show that plague epizootics are always of an acute character and greatly reduce the density of the rodent population When it comes to the state characterized by the local population as the mice have disappeared,' the epizoötic obviously stops until a new outbreak starts when the quantity of rodents has again increased This waving curve suggests either the existence of chronic forms in the inter epizootic periods or a new infection every time The former conjecture has never been corroborated, as to the latter, there is an accumulation of facts, which seem to prove its possibility These are the facts of lively intercourse which exists among rodents, and the exchange of ectoparasites, causing the spread of epidemics

The natural conditions are favourable Loose sand spots with more or less hard ground covered with vegetation are inhabited by the various rodents of which there are to be found many representatives on the steppe area such as *C mugosaricus*, *Alactaga* F cuv *Dipodipus* *Arvicola macrotes* Raddens, *Mus lagurus* Pall, etc, as well as the inhabitants of the sands *Cynomis fulvus* Licht *G meridianus* *Mus musculus* *Alactaga mesocricetus*

In the locations with transitional ground they come into contact The exchange of fleas is another proof of the close contact of the various rodents Fleas

easily change their host under experimental conditions Investigating the opened burrows of rodents in the transitional areas and their vicinity, we succeeded in finding the mouse flea (*Ceratophyllus mokrzeckii*) twice and the jerboa flea (*Mesopsylla eucta*) on susliks (*C mugosaricus*) 15 times

Mice often harbour the suslik's fleas (*Ctenophthalmus breviatus* Wag, *Ct pollex* Wag *Ceratophyllus tesquorum* Wag) In the nests of field mice were discovered about 90 per cent of suslik fleas *Ct breviatus* Wag and *pollex* and in the nests of sand mice suslik fleas (*Neopsylla setosa* Wag and *Ct pollex*, and the jerboa flea—*M eucta* together with the mouse flea—*C mokrzeckii*

The contact of rodents and exchange of fleas causes the epizootic to spread from one species of rodents to another It has been observed as a proof that as the sand mouse and the domestic mouse (*G meridianus* and *Mus musculus*, Wag) harbour two species of fleas *C mokrzeckii* and *C laeviceps* and *Xenopsylla mycerini* Roths these rodents succumb to the plague epizootic either simultaneously or one after another and as the sand mice harbour jerboa fleas (*M eucta*) both sand mice and jerboas succumb to the epidemic at once (Bukadam, *1926 27*) In this way we have a *circulus viciosus* An epizootic having obviously terminated among certain rodents breaks out among others to return to the first ones after having passed a certain circle

Very peculiar and suggestive is the mechanism of transmission of plague to man Though the epizootics do not seem to be limited to any definite season endemic outbreaks in the sandy areas usually occur in autumn This is because in autumn and the beginning of winter man comes into the closest contact with rodents At this time the nomadic kirgheez settle in their winter huts which amidst the bare sand are the only habitable places for mice Both man and mouse are also interested in a wild gramineous plant (kumarchik) which serves as a food for the kirgheez as well as for mice The period of its collection which happens to be in the late autumn, is that of the closest contact of man with rodents If there is an epizootic an epidemic is unavoidable

Such is the mechanism of the rise in the diagram If we pay attention to the ætiology of the outbreaks of this period we shall find almost exclusively sand and *domestic mice Thus plague epizootics among rodents in the South East are* associated with a definite time and place and are spread among definite groups of rodents with a definite regularity Plague epidemics break out with the same regularity They are very often connected but sometimes a very little epizootic draws first only one bubonic case in man followed by a great secondary pneumonic epidemic through direct contact This was probably what occurred in the year 1910 in Manchuria *We have sometimes also a very little epizootic in house mice in one* mud hut alone and many cases of secondary pneumonic plague In all these outbreaks there is practically no epizootic On the contrary sometimes a great epizootic draws no epidemic This occurred in the year 1924 when we had an epizootic area of about one thousand square kilometres with no human cases Why?

there was no contact of the native population with plague susliks because there was a famine and there was no work in the fields Next in a fertile year at the same places there was an epizootic and also a great outbreak

When contact has occurred and the first and one or more bubonic cases have gone down the plague outbreak takes one of two courses as is seen here

Sometimes at the end of the outbreak we observe some bubonic cases again as a consequence of the participation of human parasites Very often there is a spontaneous decline of pneumonic plague epidemics as is excellently and very truly explained by Wu Lein Teh in his ' Treatise

Consequently it may justly be said that for epidemiological and other practical reasons, the first bubonic cases are the most important for establishing the fundamental question of the plague problem how the plague virus is kept alive in nature in the endemic areas during the periods of epidemic and epizootic subsidence? A plague epidemic is the result of an epizootic which is a phenomenon of time, transitory periodically ceasing and perpetually recurring It is quite obvious that an epidemic of plague is possible *only* when there is a permanent and positive reservoir of the plague virus Where is it then?

Two answers may be given —

(*a*) The reservoirs of the plague virus are insects—fleas

(*b*) The reservoir of the plague is the animal organism

The Institute and its local organizations have been working on the solution of this problem for nearly the past four years

The fleas of the South East (those of rodents man and domestic animals) had not been investigated at all up to this time During these four years 111 collections have been gathered by collective work from a very extensive area of territory These collections contained 71 205 specimens of ectoparasites taken from 7 489 subjects The material has been carefully examined and studied Forty seven species of fleas belonging to twenty two kinds have been found In this way we have obtained a clear idea about the fleas parasitizing the rodents belonging to our district The distribution of principal species is incorporated in these tabulations This work, which cost great labour has given us a basis for the study of the role of fleas in the epidemiology of plague

Our work has been performed in two ways experiments under laboratory and field conditions, and observation and verification in the field of the data obtained experimentally

This work was peculiar in that we have always had to deal with species of fleas exactly identified zoologically

Under laboratory conditions it has been proved that all fleas in our South Eastern area, subjected to the experiment, are *able to be infected* with plague and to transmit it to healthy animals No specific ' plague ' flea like *X cheopis* of the tropics has yet been found It is, however, possible that a correction will be made by more profound studies in the future Even at present an interesting parallel has been observed, the area of the distribution of *Ct pollex* seems to repeat

the boundaries of the plague suslil area But for the while it is only a suggestion *The role of a plague' flea seems to be played by every flea of rodents*

What is this role like?

Laboratory tests have shown that suslik fleas mainly *C tesquorum N setosa* and *Ct breviatus* are not subjected to winter sleep and while their hosts hibernate they feed on the sleeping susliks If the fleas for any reason lose their host they are able to starve long enough They can live about 13 months in a cellar at a temperature from 8°C to 10°C with sufficient humidity in the field fleas have also been found alive in empty burrows at the end of winter which means that hostless and starving suslil fleas can easily live through the winter—the time of anabiosis for their host or the time of its total absence

At a room temperature of 15°C to 17°C and 92 of humidity fleas live not less than three months at a temperature from + 19°C to 25 2°C fleas also survive about three months Having been fed on an animal in a state of acute plague sepsis and then kept starving suslik fleas were found to live in the infected state in the cellar *from 20 days to one year under conditions of absolute famine and for* about seven months when being daily fed

All these fleas had plague bacilli of undiminishing virulence in their entrails

We see that at a continuously low temperature fleas can starve for over 12 months and being infected can keep alive the plague microbe under laboratory conditions from one epizootic season to another We must add that under *laboratory conditions suslik fleas (especially C tesquorum and N setosa)* readily bite man

Do fleas always contract plague when fed on an infected animal?

Always if they are fed on a dying animal with acute sepsis but not always if fed on an animal with mild sepsis a day before death Attempts to infect fleas *by feeding them on a suslil 1 to 2 days after its own infection and long before its death failed*

It was found possible to transmit plague from an infected suslik to a healthy one and to many other rodents through the bites of fleas by the mechanism advocated by Bacot and Martin

Is this transmittance easy?

Out of 25 susliks subjected to the test three contracted plague through plague-infected fleas fed on them in the quantity of three to four The percentage of infection is about 12

THUS IN CERTAIN CONDITIONS OF A LABORATORY EXPERIMENT THE RÔLE OF SUSLIK FLEAS IS VERY GREAT

Do natural conditions correspond to these? The hot South Eastern sun and drought in summer are obviously against such a supposition Meanwhile, under different conditions, the results of laboratory tests on fleas are different Infected fleas, if kept at the same high humidity, but at a higher room temperature can live for only three months At 37°C for only five days Both plague and flea are better kept alive under these conditions if they are being daily fed Then fleas can live during 28 days at 37°C, and for about 62 days at 27°C, this being the maximal duration of life for fleas under such conditions

HERE IS SUGGESTED THE POSSIBILITY TO CONSIDER FLEAS AS A RESERVOIR OF PLAGUE VIRUS

What can play a deciding rôle in the solution of this problem? Of course, only observations in the field and the finding of plague infected fleas that have lived for a long time without a host We tried to solve this problem by opening suslik burrows and investigating their contents, mostly fleas, at different seasons Only the living contents of the nest can be used to give answer of quantity and varieties of parasites at different seasons We succeeded three times in finding fleas in the field once, those of a suslik, once, fleas of a large sand mouse (*Rhombomys opimus* Licht) and, once fleas of a *M lagurus* In all three cases the findings were made under the conditions of an acute epizootic They *only* proved the rôle of fleas as disseminators of plague infection, the rôle recognized by everybody

We also *tried* to find in the field proof of the rôle of fleas as *reservoirs* of plague infection For this purpose we collected fleas in the areas of suslik plague epizootics, which were observed by us in a succession of seasons in the same places, and which might therefore be considered as stable centres of suslik plague Last summer as many as 25 per cent of rodents contracted plague Their burrows were opened during three periods directly after the onset of hibernation, in the middle of winter, and in early spring on the awakening of the susliks We can foresee an objection, which although slight is quite possible, that we might not have come across the infected burrows We however, found susliks that had been dead for different lengths of time in many of the burrows Other causes of death besides plague can almost certainly be excepted, a plague epizootic being prevalent at the time The flea population of these burrows was investigated with special care To avoid casualties we created an experimental area in a field, about one eighth of a square kilometre On half of this area with a suslik population of about 350 individuals, surrounded by a solid double ditch, we let out about 25 plague-infected susliks Plague epizootics developed during summer and only a few surviving susliks came into their burrows to hibernate It was on this area and in the above three periods that the burrows were opened and the nests of susliks investigated Their contents were

carefully gathered fleas sorted and some of them placed alive to feed on the experimental animals (white mouse guinea pig) The majority were killed by chloroform washed with sterilized water on a sifter, crushed in a mortar and the emulsion introduced subcutaneously and intraperitoneally to white mice and guinea pigs The material was also partly seeded on agar plague bacilli being sometimes accumulated in broth at the temperature of + 7°C to + 6°C

In the areas of obstinate epizootics 655 suslik nests were collected out of which 23 551 living suslik fleas were obtained In no case was the presence of plague virus in the fleas proved Plague virus was not found *preserved* in fleas either in the beginning of winter or in the middle of it or in early spring Disagreement with the laboratory data may be accounted for either by the artificiality of the tests or by the extreme scarcity of fleas preserving plague virus without an epizootic or epidemic in the field In both cases the only deduction is that the role of fleas as reservoirs of plague infection was little probable and could not be proved under natural conditions This fundamental question of plague epidemiology cannot be solved before a series of exhaustive experiments have been conducted by keeping alive for a long time undoubted plague infected fleas under strictly natural conditions

WHERE DOES THE RESERVOIR OF PLAGUE EXIST IN NATURE ?

Rodents and man this is the only answer for the present Attention must be paid to other inhabitants of the rodents burrows

SPONTANEOUS PLAGUE IN CAMELS

A peculiarity of the plague foci in South East Russia is *spontaneous plague in camels* This is illustrated by the following Table

From the year 1911 and up to the present time spontaneous plague in camels was bacteriologically proved it was *followed by 18 plague outbreaks with 139* victims In all cases the epidemic began after a plague-sick camel was killed and dressed according to Kirgheez customs

Plague in camels is experimentally proved and rouses doubts no longer

To repeat —

(1) Plague in the South East is periodic,

(2) This periodicity is dependent on —(*a*) the species of rodents carriers of plague and their biological peculiarities and (*b*) the mode of life of the steppe population coming into contact at definite periods with rodents

(3) In the suslik populated area epizootics are based on the change in susceptibility of hibernating rodents in the sandy regions—on the series of rodents involved in epizootics and exchange of ectoparasites

(4) The possibility of preservation of the plague virus by fleas for a very long time up to one year has been proved experimentally

(5) Attempts at finding plague infected fleas in the field in the absence of an acute epizootic has failed,

TABLE

Plague outl reaks originated by camels in the South Fast of U S S R. from 1899 to 1927

No.	Locality	Date	Quantity	Authors	Length of Outbreaks	Taken Sick	Died	Recovered.	Type.
	Bukeev Government								
1	Saganai	1911 August and September	2	*Deminsky*	*19th August—28th August*	18	17	1	Bubonic Pneumonic
2	Akchagil	1911 October	1	Klodnizky	27th September—7th October	12	12		Bubonic Pneumonic
3	Agjata	1911 November	1	Klodn zky	November	2	2		Unknown
4	Sar Tub	1913 November	1	Shukevich	November	1	1		
5	Ak Tube	1913 December	1	Klodn zky	13th December—7th January	4	4		Bubonic Pneumonic
6	Bulanai	1914 June	1	N kanoroff	12th May—9th June	10	10		Bubonic Pneumonic
7	Man sl chag l	1914 June	1		.6th May—18th June	6	6		Bubonic
8	Ulken chagil	1915 July	1	N kanoroff	?0th May—21st June	33	33		Bubonic Pneumonic
9	Marscleu	1917 December	1	N kanoroff	20th December 26th December	4	4		Bubon c Pneumonic
10	Aizarl	1923 November	1	Golov	20th November—15th December	1	1		Bubonic
11	Din Kzil	19 3 December	1	Golov	17th December—17th January	1	1		Bubonic

Etiology: All cases are assoc ated with the killing of sick camels and the dressing of their carcasses

TABLE—*concld*

No	Locality	Date	Quantity	Authors	Length of Outbreaks	Taken Sick	Died	Recovered	Type
12	Hoton Tasta Kalmuch District	1924 November	1	Suvorov and Kniazevsky	23rd November—31st December	15	14	1	1 Bubonic 14 Pneumonic
13	Locality, Mambet Niaz *chagli* *Transcaspian* District	1926, November	1	Tgnatov	18th November—16th December	2	1	1	Bubonic Pneumonic
14	Locality, Tienali, Trans caspian District	1926, November	1	Tgnatov	1st December—22nd December	3	1	2	Bubonic
15	Kirgheez camps of Kras noyar region Astra khan Government	1926, November	1	Suvorov	4th November—7th January	5	4	1	Bubonic
16	Jankara, Bukeev Govern ment	1927, April	1	Taller	14th April—1st June	11	9	2	Bubonic
17	Locality, Egizlak, Trans caspian District	1927, August	2	Kluchevsky	2nd August—30th August	10	7	3	Bubonic

Ætiology All cases are associated with the killing of sick camels and the dressing of their carcasses

(6) Organisms of animals and man serve as reservoirs of plague

(7) An epidemiological peculiarity of the South East is plague in camels

Such is the epidemiological basis for the organization of anti plague work in the South East

If on the one hand epizootics are periodic if on the other hand the contact of the population with plague sick rodents is regular and periodic if in consequence plague outbreaks are periodic the basis of all our practical plague work is quick discovery and investigation of all places where the epizootic can be and the constant observation of the state of the rodents This is the work of the flying investigating detachments and local laboratories

On the discovery of an epizootic a vaccinating campaign and sanitary educational work should be carried out among the local population to prevent its contact with rodents At the same time a temporary ambulatory laboratory is organized to discover the first cases of plague If the plague outbreak occurs the staff of the detachments and laboratories directs all the plague work having the guiding instructions Possible contact of the population with rodents should be broken off by partial or total extermination of the rodents This is quite impossible in the semi desert areas We know that when an intensive campaign is started the area in a short time again becomes infested with rodents These occupying infected burrows start an epizootic again

During the last season we have made attempts to fight the plague endemic by making the area healthy In a certain area infected with plague epizootics (some natural boundaries as a river lake etc are desirable) the total extermination of the rodents—the carriers of plague has been carried out At the same time we tried to kill with chlorpicrin the whole of the living population in their burrows disinfecting the whole locality If later on rodents from the neighbouring healthy regions should settle in this locality there will be no cause for a plague outbreak

Of course such work is only possible on a limited scale with favourable natural conditions

We succeeded in liquidating the plague endemic in the area of Astrakhan by these methods

Such a plan of practical plague work based on the data of many years experiments and observations on the biology of rodents was only devised when all the scientific and practical plague workers in the South East concentrated at the Microbiological Institute in Sarotow in the year 1923

Every year we have one anti plague conference The last one was this year the All Russian Plague Congress Our plan of work was accepted as the correct one and now it is indeed the model for the work in Turkestan and Transbaikalia

TREATMENT OF BUBONIC PLAGUE IN INDIA.

BY

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AND

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INTRODUCTION

ALTHOUGH the value of Haffkine's plague prophylactic as a personal measure of protection against an epidemic of plague has been well established and the demand for the prophylactic is steadily on the increase in India, yet the results, which have followed the several methods of treatment hitherto employed, have been disappointing

This paper deals with the several lines of treatment employed in India and the results we have obtained last year from the use of D'Herelle's 'anti pestiphage'

All the methods of treatment for plague have been either symptomatic or specific and these may conveniently be grouped under the following heads —

(A) Surgical, (B) Medicinal, (C) Plague vaccines, (D) Anti plague sera, and (E) 'Anti pestiphage' There is yet another which needs but a passing notice, namely, Organotherapy

Organotherapy — In the November number of the *Haleem and Vaidyan* of 1924 Khan Bahadur Hakeem Moulvi Hajee Abdur Raheem Sahib, Assistant Superintendent Survey of India Bangalore, recommended as a sovereign remedy tiger s flesh dried powdered and administered in the dose of a pinch mixed with honey This would produce, in his opinion, a radical cure in a case of plague

(A) SURGICAL TREATMENT

Operative treatment of plague consists in aspiration, incision or excision of the bubo In the early days of plague in India some surgeons have either incised or excised the bubo in acute cases with disastrous results The modern practice of treating the bubo among the lay public consists in blistering branding or leeching the bubo in acute cases It seems a drastic method, for we have seen very foul and indolent ulcers following on blistering and extensive necrosis following on branding Most medical men now either aspirate the contents of a suppurated bubo or incise it when the acute stage has passed, that is about the tenth day of the disease

(B) MEDICINAL TREATMENT

Several drugs are employed for the treatment of plague, these may be grouped under two heads (1) those employed to relieve the distressing symptoms which

accompany the disease and (2) those employed to effect the destruction of plague bacilli in the body

The symptomatic treatment consists in an effective combination of drugs with definite pharmacological action, which is usually administered by mouth, according to the various systems involved We have summarized the line of treatment employed by Choksy at the Maratha Plague Hospital, Bombay who has had exceptional experience in the treatment of plague extending now over a period of 30 years

Fever is controlled by the application of ice on the head, by cold and tepid sponging, or by cold and tepid wet pack to the trunk only

The circulation is supported by absolute rest in bed in the recumbent position immediately on the first signs of fever and pain in the bubo and this is enforced for at least three weeks in every case

Cardio vascular paresis is treated with a mixture containing the following —

℞	
Sol adrenaline chloride	
vel	
Ext renaglandin	m 10—30
Sparteın sulph	gr ½
Liqr strychnine	m 3
Glycerine	m 30
Aquam	ad oz ½—1

and administered every two hours day and night the patient receiving on an average 9 to 10 doses in 24 hours the maximum dose of supra renal preparations being determined by its effect and kept up for at least 3 to 4 days and if the circulation is well sustained the dose is gradually reduced at first and later administered less frequently In cases where the supra renal preparations fail to give satisfactory results liq atropin m ½ to 1 is substituted in the above recipe If *meningeal irritation* is present strychnine is withheld

Any increase of the respiratory ratio that does not correspond to the existing temperature or pulse rate, or if the number of respirations exceed 30 per minute early and free counter irritation to the chest with tinct iodi and hot poultices or antiphlogistine is indicated to guard against the two most fatal complications, namely, terminal pneumonia and subacute pulmonary œdema If cough is troublesome, stimulant expectorants with senega or squill are administered Should sputum be copious and hæmorrhagic, calcium chloride in gr 20 doses is given 3 or 4 times a day with or after feeds, calcium lactate may be similarly employed The mouth should be kept scrupulously clean the lips gums and teeth should be attended to with liberal applications of boro glycerine or lemon juice and glycerine

Constipation is a common accompaniment in this disease and is best relieved by a simple enema, repeated daily or every other day, as needed Irritability of the stomach yields readily to 10 to 30 minims of mist pepsin bismuth or cocaine

hydrochloras gr 1 10 to $\frac{1}{8}$ in a teaspoonful of iced water given every half hour for 3 to 4 doses, followed by iced soda and milk in very small quantities, should it persist, all nourishment by mouth is stopped and rectal feeding resorted to Chloretone in gr 10 doses is also efficacious in such cases Hiccough is another troublesome complication best treated with an ice bag or sinapism over the epigastrium or painting a broad band of iodine over the neck along the course of the pneumogastrics, but failing this, hypodermic injection of ether or morphine, gr $\frac{1}{8}$ to $\frac{1}{6}$ or morphine gr $\frac{1}{8}$ and atropine gr 1/150 to 1 100 may be required Tympanitis if severe is a serious factor in prognosis and if the condition of the patient will allow, gr 5 of calomel with an equal quantity of sodium bicarbonate with some carminative should be given, milk must at once be withdrawn and thin arrowroot conjee substituted Enemas of *turpentine (ol terebenth, oz* $\frac{1}{2}$ to 1 white of one egg and thin starch gruel 1 pint) should be administered every 6, 8 or 12 hours If these measures do not give adequate relief, salol, gr 15, or turpentine (ol terebenth, spiritus etheris nitrosi, and spiritus chloroformi minims 10, with mucilage and water) should be administered and persisted with *Turpentine stupes or ice cold compresses to abdomen occasionally give* relief Diarrhœa if terminal does not respond to treatment, in its early stages it must be checked immediately Bismuth, dermatol, salol or hydrar cum creta are all useful and occasionally an addition of grs 2 to 3 of Dover's powder enhances their action

Delirium is the most important complication of the nervous system and physical restraint by strapping the patient down to the bed and the hypodermic injection of hyoscine hydrobromide in doses of gr 1/100 to 1/75 are indicated, at least 4 hours must elapse before the injection is repeated, if found necessary Morphine should be the last resort if the former has failed to subdue the delirium The patient under the influence of *hyoscine or morphine should be kept constantly under oxygen until* the circulation improves and he gets out of the enforced sleep refreshed In milder cases ammonium bromide in gr 10 doses with drm 1 of tinct hyoscyami or chloretone in doses of grs 10 to 20 has been found most useful Atropine has been found very beneficial in association with the bromides General nervous prostration in the acute stage *requires the hypodermic injection of camphor oil (camphor 2, ether* sulphuric 3 and olive oil 7 parts) in 20 minim doses repeated every 2 or 3 hours

Pain and tenderness in the bubo is a matter of primary importance and is relieved by heat and belladonna applications

The *antiseptic treatment* (chemo therapy) aims at the destruction of plague bacilli *in the human body* With this end in view, Choksy introduced the method of injecting germicides into buboes during the first epidemic of plague in Bombay (1896) using pure carbolic acid and also equal parts of acid and liquor iodi, 10 to 20 minims were injected into each bubo by a hypodermic syringe, the injection being repeated once only after 24 hours He believed that this method of dealing with *buboes in the early stages* is capable of wider application Since then many drugs of known germicidal value have been injected not only subcutaneously into

the bubo, but also intravenously Some of these are (1) *Formalin and its compounds*—Roehr (1912) suggested the injection of a two per cent solution of formalin into the bubo, and Deggeller (1915) treated cases of plague with intravenous injections of formaldehyde sodium bisulphite ('Formbisit') and considered that the results obtained were promising (2) *Salvarsan and allied arsenical compounds*—Lancelin (1912) employed intravenous injections of salvarsan without success Aumann (1912 13) found that intravenous injections of salvarsan had no favourable influence on the course of the disease Ram Mansoor and Simpson (1922) treated cases of plague by intravenous injections of neosalvarsan and claimed good results Schut (1921) treated cases of plague with intravenous injections of neosalvarsan and all ended fatally Marshall and Acchuru Ram (1922 23) treated cases with neosalvarsanobilin and neokharsivan and found that the latter was a better drug of the two (3) *Electrargol*—Denman (1914) found that early cases of plague treated by intravenous injections of electrargol were benefited In his series of cases the mortality among the treated was 60 4 per cent while among the controls it rose to 83 4 per cent But Ilvento and Mazzitelli (1914) on the other hand considered that treatment with electrargol in plague was not of much value (4) *Eusol*—Connor (1916) suggested the subcutaneous injection of eusol and Brayne (1917) found that intravenous injections of eusol in plague had no influence on the course of the disease Baker (1920) also treated cases of plague in Uganda with eusol with no benefit Patel (1926) employed *Izal* in the treatment of plague without benefit (5) *Mercurochrome 220 soluble*—Andrew Balfour (1924) suggested the use of mercurochrome in plague, he also reported that he had received accounts of a few cases of plague in which the drug had been distinctly beneficial We (1926) found that in experimentally infected animals namely rats and rabbits injections of mercurochrome in sub lethal doses either single or repeated had no influence on the progress of the disease or its termination, Patel (1926) also found that human cases treated with mercurochrome at the Maratha Plague Hospital, Bombay were not benefited At the Haffkine Institute, one of us (B P B N) tested the therapeutic value of the synthetic preparation of a compound of mercury with trypan blue on rats and rabbits experimentally infected and found it has no beneficial effect on the disease (6) *Iodine and its compounds*—Booth Tucker of the Salvation Army was the first to introduce the oral administration of tinct iodi in the treatment of plague and claimed satisfactory results Connor (1912 13) employed tinct iodi intravenously in the treatment of plague and his three cases recovered under treatment Liston (1913) studied the efficacy of this treatment at the Maratha Plague Hospital by determining the severity of infection by bacteriological examination of blood of his cases and found that intravenous injection of tinct iodi in 20 cases resulted in the death of 17, while of the 20 controls under ordinary treatment 16 had died The examination of the blood of over 500 cases of plague patients showed that all persons with a larger number of plague bacilli than ten per ¼ c c of blood drawn from the vein at the elbow and cultured on agar invariably died, while a proportion of cases with no plague bacilli in the blood or containing less than ten plague bacilli

in ½ c c recovered Therefore excluding the invariably fatal cases with many plague bacilli in their blood before treatment, he had 4 recoveries out of 9 cases among the controls and 8 recoveries out of 12 cases treated with iodine by the mouth and 3 recoveries out of 10 cases treated intravenously The number of cases treated in each series was not sufficiently large to allow of any definite conclusion Rama Iyer (1916) treated 6 cases with intravenous iodine with 1 death, Acchuru Ram (1917) treated 103 cases by the above method with 87 deaths Mullanna (1920) suggested the use of iodine combined with camphor and thymol and Subbiah Pillai (1921) treated 3 cases by this method with 2 deaths Vassallo (1921) treated 20 cases with iodine and had 4 deaths Jendwine (1923) reported that Hari Ram had obtained with treatment of intravenous iodine good results of the 28 cases treated there were 2 deaths and these were early cases Pal (1924) treated 2 cases with no death by this method Naguvie (1924) treated 100 cases with iodine and of this number 25 died Bharadwaj (1926) treated 100 cases with iodine with 20 deaths Grimes (1926) treated 7 cases of plague with colloidal iodine and of this 1 died In 1926 we saw a large number of cases of plague being treated with iodol (a French preparation) at the Plague Hospital, Secunderabad Deccan and the medical officer claimed 50 per cent cures in cases of plague As intravenous injection of iodine in plague is a popular measure with the medical profession in India, it seems desirable that controlled experiments on the human on a large scale to determine the limitations of this method of treatment should now be undertaken

(C) Vaccine Treatment

Row (1905) found that the local infection of the plague bubo with a species of *Staphylococcus pyogenes* was attended with beneficial results Of the 28 cases treated 18 recovered with a case mortality of 35 7 per cent

Row and Turkhud (1907) studied the effects of plague vaccines, whose toxicity had been modified by one or other of the laboratory methods in the treatment of plague with the following results —

Table I

Nature of vaccine	Cases treated	Deaths	Percentage mortality
Salted vaccine	40	29	72 5
Detoxinated by the precipitation method '	13	10	76 9
Old detoxinated vaccine	17	14	82 3
' Composite vaccine	10	9	90 0
Composite but treated for 2 hours at 60 C	16	10	62 5
Total cases	96	72	75 0

Row (1913) studied the curative value of a '*glycerinated vaccine*' in plague and found that in doses of 85 to 90 million bacilli it had a distinctly beneficial action in cases of plague not actually septicæmic He recorded a recovery per cent of 83 6 in his treated cases Chunmaja Vspishka (1924) treated 17 patients with glycerinated vaccine in doses of 100 millions of bacilli with 3 deaths Nikanoroff (1924) treated cases of plague with glycerinated vaccine and found the results not encouraging Stocker (1924) employed sensitized vaccine in the treatment of plague in the Mardan *Epidemic and of the 17 cases (uninoculated) treated there were 5 deaths with a* percentage mortality of 29 0, while the 18 (uninoculated) controls had 11 deaths with a percentage mortality of 61 0 Patel (1927) found the sensitized vaccine of little benefit among his patients at the Maratha Plague Hospital The combined use of the vaccine and the serum has also been employed with varying results

(D) Serum Treatment

Following on the outbreak of bubonic plague in China Yersin (1896) prepared an anti plague serum on the lines employed for the manufacture of anti diphtheritic serum by Behring and Kitasato and found that when this serum was injected into plague patients it reduced their mortality from some 90 per cent down to 7 per cent The preparation of such a serum required many months of continued treatment of horses from which the serum was to be obtained These results were so striking, that the Health Department of the municipality of Bombay began with the pre paration of anti plague serum in November 1896 under the direction of Haffkine who for this purpose immunized horses cattle goats and sheep and thought that the serum of immunized sheep was the more promising In February 1897, he tested the serum on 6 patients, out of the 4 cases treated within 24 hours of the attack, 3 survived *From these results he concluded that there was the possibility* of the anti plague serum rendering service in the treatment of the disease and that it was necessary to start treatment within the first 24 hours As a large number of repeated injections were required with the serum he employed he considered the possibility that with further treatment of animals the anti toxic power of his serum would increase so as to effect an influence on the older cases and with smaller doses, therefore this serum was not issued for general use

Lister held so high an opinion of the efficacy of Yersin s treatment that the Secretary of State for India desired that steps should be taken to give full trial to Yersin's system of treatment Yersin therefore arrived in India in February 1897 and proceeded to Annam to prepare the serum He came to Bombay in March and started treating a considerable number of private cases Out of the 50 cases treated by Yersin 17 died with a mortality of 34 per cent of these 17 were treated on the first day of illness with 2 deaths 17 on the second day of illness with 6 deaths and 12 on the third day of illness with 6 deaths Yersin stated that this serum was hastily prepared and therefore less active than the one he had employed in China and that, in consequence, larger doses had to be employed.

Further trials with several anti plague sera have been carried out since then both in India and elsewhere

We have here summarized the results of treatment with anti plague serum obtained in India

TABLE II

RESULTS OF ANTI PLAGUE SERUM IN THE TREATMENT OF HUMAN PLAGUE

Indian experience during the years 1897—1911 *with four sera largely used*

Serum	STRICTLY COMPARABLE SERIES						NOT STRICTLY COMPARABLE SERIES					
	TREATED			CONTROLS			TREATED			CONTROLS		
	Number	Deaths	Percentage died	Number	Deaths	Percentage died	Number	Deaths	Percentage died	Number	Deaths	Percentage died
Roux Yersin	226	168	74 3	231	163	70·5	309	166	53 7	260	200	76 9
Lustig	608	436	71 7	609	48[illegible]	79 1	943	548	58 1	2 145	1 713	79 8
Terni	110	89	80 9	110	90	81 8	2	2	100·0			
Brazil	70	58	82 8	70	60	85 7	2	2	100 0			

For details *see* Tables A B C D and F (pages 109 to 113 and 122 123)

TABLE III

ANTI PLAGUE SERUM IN THE TREATMENT OF HUMAN PLAGUE

Summarized Results

(From 27th April to 3rd June 1908 *from 15th March to 10th May,* 1909 *from 15th March to 14th May* 1910, *from 20th January to 28th May* 1911 *and from 29th February to 25th March,* 1912)

Bacteriological Diagnosis Blood	TREATED CASES		CONTROL CASES		REMARKS
	Number	Deaths	Number	Deaths	
+++	81	81	100	100	
++	18	18	24	24	Moderate and severe septicæmic cases
	99	99	124	124	
+	49	35	35	20	Slightly septicæmic cases
—	90	24	78	24	Clinically plague cases
	238	158 (66 3 per cent)	237	168 (70 8 per cent)	Total number of cases

Clinically

(1) Serum modified the course of the disease

(2) Life was prolonged

(3) Bacteria tended to diminish in numbers in the circulation, becoming localized in the tissues where they caused necrotic nodules and abscesses

(4) *A reduction in the degree of septicæmia occurred in 53 of the serum* treated cases and in only 30 of the controls

(5) It would seem that in a case that was hanging in the balance between recovery and death the serum fortified a little the resisting powers of the patient

(6) Results from serum treatment are by no means encouraging

(L) Bacteriophage in the Treatment of Human Plague

D Herelle in 1925 treated four cases of bubonic plague at Alexandria with a bacteriophage originally isolated in Indo China in 1920 from the fæces of a rat Of these three cases recovered These results were brought to the notice of the Government of India and instructions were issued to test this bacteriophage prepared by him at Alexandria on human cases of plague in India

The bacteriophage arrived in Bombay about the beginning of March 1926, we were deputed to carry out the test with the co operation of the local medical authorities at Hyderabad (Deccan) and at Agra Between the 16th of March and the 30th of April we studied the results of treatment on 103 cases with 97 controls

Bacteriological Diagnosis of Human Plague—Clinical diagnosis of plague is not attended with any great difficulty especially during an epidemic as fever bubo (painful) furred tongue (dry and brown) injected eyes drunken gait thickness of speech all taken together are sufficiently characteristic of the disease But not *infrequently* cases of mumps septic and venereal buboes cerebral malaria and influenza have been mistaken for plague during this epidemic

Bacteriological diagnosis is made by the presence of plague bacilli in the bubo and in the blood The technique is simple with a sterile hypodermic syringe containing a few drops of saline or broth puncture is made into the bubo the fluid in the syringe is injected into the substance of the bubo and withdrawn into the syringe the bubo juice thus obtained is cultured on an agar slope and a smear is made on a slide for immediate examination To inject a few drops of sterile fluid into the bubo is an advantage as it is sometimes hard to draw any material from the bubo into the dry syringe it is also an advantage to culture the bubo juice as the smear may occasionally fail to reveal the presence of plague bacilli, if the organisms in the smear are few and if the preparation is overstained the characteristic appearance of the organism is lost At the same time a blood culture from the vein is made on agar and in broth For this purpose a vein at the bend of the elbow is selected and a small quantity of blood sufficient to cover the surface of two agar slants (about a quarter of a c c) and about half a c c to inoculate the broth is withdrawn by another sterile syringe and the contents are distributed on the surface of two agar slopes and into a broth tube the cultures are left in the dark at room

temperature to incubate and are examined at intervals of every 24 hours The growth of *B pestis* is rapid and even before the growth is visible to the naked eye, smears made from the cultures reveal the presence of *B pestis* within some hours of inoculation

Treatment with Bacteriophage—Every case submitted for study was examined bacteriologically by the above technique to establish a correct diagnosis and every alternate case was treated with the bacteriophage The procedure recommended by D'Herelle was as follows —

'As soon as possible after the appearance of the bubo inject the contents of one ampoule that is, 1 c c into the centre of the bubo, if there are two or more buboes, inject a half c c into each one, if the treatment is begun late, it may be necessary to administer a second injection 24 hours after the first has been given and some times a third injection again 24 hours later If on the day following the first injection, the temperature should after having dropped begin to rise again, ad minister on the third day a second injection of 1 c c, if the temperature falls gradually after one injection do not make further injections, a second or a third injection should only be given when the temperature remains stationary or is on the ascendency Until now only cases of bubonic plague have been treated with bacterio phage, in the case of septicæmic or pneumonic plague, the bacteriophage suspension should be tried intravenously'

Cases studied differed as regards the duration of illness and the appearance of the bubo, a considerable number of cases were clinically septicæmic in their appearance and so these cases were treated on their clinical appearance, awaiting the bacteriological diagnosis Every treated case which had a bubo received one ampoule of the bacteriophage into the centre of the bubo and the contents of one ampoule intravenously According to the clinical features from day to day, these doses were repeated in some cases at intervals of 24 hours The number of doses varied from one to six

The results are tabulated on the findings by bacteriological examination in each case, as statements made by patients or their friends regarding the duration of illness or the appearance of bubo could not be relied upon

The presence of plague bacilli in smears or cultures made from the bubo juice (or even from the site at which much pain is complained of before the bubo is defi nitely felt) confirms the diagnosis of bubonic plague The presence of plague bacilli in blood in the presence of a bubo indicates that the case has become septi cæmic, and in the absence of a bubo that it is septicæmic in type The absence of plague bacilli either in the bubo or blood, with repeated examinations indicates that we are dealing with a case which is not plague The blood cultures on agar have a further advantage in this that they afford a measure of the severity of the septicæmia present, according to the number of colonies developed on the two agar slants the intensity of infection is arbitrarily divided into (*a*) severe that is, those with a hundred or more colonies, (*b*) moderate, that is, those with ten or more colonies but less than a hundred, and (*c*) slight, that is, those with one to ten colonies

Prognosis largely depends on the severity of infection, as cases of moderate and severe degree of septicæmia do not recover, while a very small percentage of cases with slight degree of septicæmia do recover A considerable number of cases which are purely bubonic in character tend to recover under hospital treatment

TABLE IV

BACTERIOPHAGE IN THE TREATMENT OF HUMAN PLAGUE

Summarized Results of 1926

Bacteriological Diagnosis		Treated Cases		Control Cases		Remarks.
Bubo	Blood	Number	Deaths	Number	Deaths	
+	+++	29	29	29	29	
	+++	3	3	6	6	
+	++	4	4	3	3	Moderate and severe septicæmic cases. (Series I)
—	++	2	2	3	3	
-		38	38	41	41	
+	+	14	11	9	6	Slightly septicæmic cases.
—	+	4	4	2	1	
		18	15	11	7	
+	—	40	12	20	6	Purely bubonic cases
—	—	7	1	16	9	Clinically plague cases.
		103	66 (64 per cent)	97	63 (65 per cent)	Total number of cases

For details, *see* Tables E and F (pages 113 to 123)

Conclusions · (1) Septicæmic cases of moderate or severe degree did not recover whether treated with the bacteriophage or not.

(2) Treatment with the bacteriophage did not influence the course of the disease and the case mortality, even in cases slightly septicæmic or purely bubonic in character

D'Herelle's Commentary

'This bacteriophage was isolated in Indo China in 1920 from the fæces of a rat and has been subjected from that time to about 200 passages Its virulence was not very high as it allowed constant "secondary cultures" to develop These secondary cultures are due to resisting bacteria The bacteriophage used in the treatment of cases in Egypt in 1925 was prepared as follows —

Every case was treated with a recently prepared bacteriophage In the first case the bacteriophage was added to a suspension of a strain of *B pestis* isolated from the patient and the resulting lysate was filtered and employed The *succeeding cases were treated with lysates, each obtained by the action of the* bacteriophage on a culture of the bacillus obtained from the immediately preceding case

It is possible that the differences in the results may be due to the fact that for the cases in India the bacteriophage employed was six or seven weeks old But I am inclined to think that the reason for this failure is really due to the fact that the bacteriophage employed was not sufficiently potent to deal with the *strains of B pestis in India which seem to be extraordinarily virulent*

We know that bacteria can acquire resistance against an insufficiently powerful bacteriophage and a culture obtained by Dr Avari from a patient three days after the administration of bacteriophage completely resisted the action of the bacteriophage

In view of these considerations, I recommend that researches be made in India with the object of obtaining a bacteriophage able to produce complete lysis of a 24 hours' virulent culture of *B pestis* (local strains) at a temperature of 38°C to 40°C within the shortest time of contact (4 to 6 hours)

As bacteriophage virulent for *B pestis* is found in the intestinal contents of rats which resist infection with plague and also in the intestine and in the buboes of men during recovery from plague a virulent bacteriophage should be isolated from these sources, but of the two the human source seems preferable'

It would be necessary for this study to examine the contents of the bubo and intestinal contents in man daily from the commencement of the disease to the period of recovery, as the presence of bacteriophage is often transient'

'From the results obtained by myself and many others in the treatment of various other diseases with bacteriophage, it is reasonable to expect that similar results will follow in plague In a closely allied disease, namely, hæmorrhagic septicæmia in buffaloes a single injection of 30 to 40 c cs of a very potent bacteriophage (able to produce complete lysis in 3 hours) resulted in prompt recovery, while natural recovery never occurs in this disease'

TABLE V

DR AVARI'S RESULTS ON THE ISOLATION OF BACTERIOPHAGE FOR *B pestis*

(Haffkine Institute Bombay December 1926 to November 1927)

(A) From Passage rats (B) Control rats (C) Immunized rats, (D) Epizootic rats (E) Rat droppings and (F) Human cases

Rat Number	Interval between infection and the killing of rat for examination	ISOLATION OF BACTERIOPHAGE PRESENT COMPLETE LYSIS IN		Bacteriophage not isolated
		4 to 6 hours	24 hours	
(A) From Passage rats				
470	9			0
587	9	(1) + (Liver and Spleen)		
644	9	(2) + (Liver and Cervical glands)		
795	8			0
800	9			0
816	8			0
827	9			0
834	8		+ (3)	
850	9		+ (4)	
852	9			0
853	10			0
867	9	(5) + (Spleen Liver, Bubo)		
876	8			0
896	8			0
899	8	(6) + (Spleen)		
901	13			0
905	9			0
907	10½			0
919	10			0
970	9			0
980	8			0
1000	8			0
1087	8			0
1132				0
1198	7			0

TABLE V—*concld*

Rat Number		[illegible] 4 to 6 hours	[illegible] hours	Bacteriophage not isolated
(B) Control rats of Dr Naidu's experiments				
Number of rats examined 2	10			0 (in all rats)
(C) Immunized rats of Dr Naidu's experiments				
35	15 to 17			0 (in all rats)
(D) Experimental survivors				
6		(10) + (Spleen in two) (11) 2 hours		0 (in four rats)
(E) Rat droppings				
2 examinations				0 (in both)
(F) Human Plague cases				
No 399 (10th day of infection)				0 (in fæces and bubo six examinations)
No 710 (slightly septicæmic)				0 (in bubo)

CONCLUSIONS

Although the value of chemotherapy in bacterial diseases is much disputed and has not yet been demonstrated in well controlled human cases it is generally admitted that specific therapy offers the greatest measure of success in bacterial infections

Although it must be admitted that the anti plague sera now in use and the bacteriophage of D Herelle of 1926 have failed to fulfil our expectations *still our* present knowledge on the production of more potent anti sera and the recent recommendations of D Herelle above referred to towards the production of a more potent

bacteriophage make it imperative that intensive researches on the specific therapy of plague should be undertaken now in India where so many human lives are lost every year due to this fell disease

APPENDIX

TABLE A

LUSTIG S ANTI PLAGUE SERUM

(G Polverini M D Municipal Laboratory Parel 7th November 1899)

Number of horses treated for the product on of serum at	Number of patients treated	Deaths	Recovery percentage	Period of observation
Florence 5	257	145	43 57	19th March to 31st October 1898
Bombay 5	218	143	34 40	1st February to 31st May 1899

Hypodermic Injections of Serum in the Treatment of Human Plague

Places where cases were treated	SERUM TREATMENT			WITHOUT SERUM			CITY OF BOMBAY		
	Number of patients treated	Deaths	Percentage recovery	Number of patients treated	Deaths	Percentage recovery	Attacks	Deaths	Percentage recovery
Arthur Rd Hospital	403	249	38 21	1 190	957	19 57	24 752	21 193	14 3
Maratha Hospital	28	17	39 28	3 378	2 73	19 12			
Mod khana Hospital				1 384	1 089	21 31			
Government House									
Parel	12	9	25 00						
Private	32	13	59 37						
TOTAL	475	288	39 36	5 952	4 778	19 72	24 752	21 193	14 37

TABLE B

RESULTS OF ANTI PLAGUE SERUM IN THE TREATMENT OF HUMAN PLAGUE

	STRICTLY COMPARABLE SERIES TREATED BY THE "ALTERNATE" METHOD						NOT STRICTLY COMPARABLE SE[RIES] WHERE SOME METHOD OF SELECTION WAS EMPLOYED				
	TREATED CASES			CONTROL CASES			TREATED CASES			CONTROLS	
	Number	Deaths	Percentage died	Number	Deaths	Percentage died	Number	Deaths	Percentage died	Number	Deaths
Yersin's Serum Himself							50	17	34 0		
1897 Others							31	19	61 2		
German Com							26	13	50 0		
Russian Serum 1897 Russ Com	50	40	80·0	50	40	80 0					
Haffkine's Serum 1897 Himself	100	?	?	100	?	? 14					
Roux Yersin's Serum *1897 Mason*							*100*	*59*	*59 0*	*100*	*83*
1898–99 Simond							10?	58	56 8	74	55
1898 99 Ind Pl Com							49	*31*	*63* –		
1899 Turkhud	28	23	82 1	28	24	85 7					
190? Mayr	31	?9	*93 5*	*31*	?9	*93 5*					
1904 West	?8	45	66 1	?8	41	60 ?					
1906—08 Choksy etc							1 081	?37	49 6		
Lustig's Serum 1897 Himself							30	6	–0 0		
1898 Clemow	13	10	77 0	13	?	?					
1898 Galeotti							*257*	*145*	*56 4*	*75?*	*59?*
1899 Polverini							403	249	61 7	1 190	957
1898—1901 Maratha Hosptl							66	49	74 2		
1901 Maratha Hosptl							44	31	70 4	?03	161
and Choksy's private cases							130	58	44 6		
1900 Modikhana Hosptl	66	54	81 8	66	48	7 7					
190? Mayr	31	31	100 0	31	?0	93 5					

TABLE B—*concld*

	STRICTLY COMPARABLE SERIES TREATED BY THE ALTERNATE METHOD						NOT STRICTLY COMPARABLE SERIES WHERE SOME METHOD OF SELECTION WAS EMPLOYED					
	TREATED CASES			CONTROL CASES			TREATED CASES			CONTROLS		
	Number	Deaths	Percentage died	Number	Deaths	Percentage died	Number	Deaths	Percentage died	Number	Deaths	Percentage died
1902 Poona Hospital	27	21	77 7	28	20	71 4						
1899—1900 Poona Hospital	484	330	68 1	484	385	79 5						
1899—1901 Parsi Hospital and Modikhana Hospital							9	6	66 6			
Yersin's Serum 1901							2	2	100·0			
190[illegible]—03 Modikhana Hospital	110	89	80 9	110	90	81 8						
Brazil's Serum 190[illegible]							2	2	100·0			
1904 Maratha Hospital and Modikhana Hospital	70	58	8[illegible] 8	70	60	85 7						
Tavel's (Berne) Serum Choksy							28	18	64 [illegible]			
Paltauf's (Vienna) Serum Choksy							8	4	50·0			
Japanese Serum Choksy							4	1	25 0			
Lister Institute's Serum 1909—11 Liston	[illegible]	147	66 1	[illegible]	163	73 4						
TOTAL NUMBER OF CASES TREATED	1 [illegible]00	877	73 0	1 188	9[illegible]9	78 [illegible]	[illegible]33	1 [illegible]99	55 7	2 319	1 8[illegible]1	[illegible]8

Table C.

Showing the Mortality Rate according to Duration of Illness at the Time of Treatment

(*From Choksy*)

Duration of illness	Yersin's Results, 1897, Bombay			Hospital Results with Sera, Bombay, 1897–1904			Choksy's Results, Bombay, 1897–1908			Controls untreated in hospitals		
	Number	Deaths	Percentage died	Number	Deaths	Percentage died	Number	Deaths	Percentage died	Number.	Deaths	Percentage died
1st day	17	2	12 0	12	7	58 3	345	106	30 7	17	10	58 8
2nd day	17	6	35 0	74	63	85 1	401	211	52 6	60	44	73 3
3rd day	12	6	50 0	62	44	70 9	306	183	59 8	76	63	82 8
After 3 days	4	4	75 0	86	67	77 9	197	115	58 37	81	60	74 0
Total cases	50	17	34 0	234	181	77 3	1,249	615	49 23	234	177	75 6

Table D

Comparative Tables.

(*From Choksy*)

I Results obtained with Roux Yersin's serum, 1905—08, among cases selected for treatment.

Treated			Controls		
Number of Cases	Deaths	Percentage died	Number	Deaths	Percentage died
380	215	56 5			
200	127	63 5	200	148	74 0
Total, 580	342	58 9			

II Results among cases rejected as unfit for serum treatment or control cases, 1905—08

REJECTED			
Cases	Number	Deaths	Percentage died
Between 1st to 5th day	572	556	97 2
6th to 9th day	185	111	60·0
10 days and over	110	51	46 3
TOTAL CASES	867	718	82 8

III Results of serum treatment in hospital and in private practice brought up to November 1908

TREATED			
Cases	Number	Deaths	Percentage died
Hospital	755	424	56 1
Private	604	221	36 5
TOTAL CASES	1 359	645	47 4

TABLE E

ANALYSIS OF CASES TREATED WITH BACTERIOPHAGE

Series I

Moderate and severe septicæmic cases

Bacteriological Diagnosis		Case	Duration of disease—days	Site of bubo	Number of treatments at intervals of 24 hours	Results	
Bubo	Blood					Death in days from the onset of the disease	Recovery
+	+++	565	4	Femoral	3	6	
		Naras	1		2	2	
		506	7	Inguinal	1	8	
		183	3	Cervical	1	4	

TABLE E—*contd*

Bacteriological Diagnosis		Case	Duration of disease—days	Site of bubo	Number of treatments at intervals of 24 hours	Results	
Bubo	Blood					Death in days from the onset of the disease	Recovery
		185	3	Femoral	1	4	
			2	Inguinal	1	6	
			5	Cervical and axillary	1	5	
			2	Inguinal	1	2	
			4	,	1	5	
			3		1	4	
			2	,	2	4	
			1		1	2	
			2		1	2	
			3	Cervical	1	4	
			2	Inguinal	1	3	
			2	Axillary	1	3	
			3	Inguinal	1	3	
			1	Femoral	1	2	
			2		1	3	
			2	Inguinal	1	2	
			4		1	4	
			3	Femoral	1	5	
			3		1	5	
		1	3	Inguinal	1	4	
		3	2	Femoral	1	3	
		18	3	Axillary	1	3	
		21	2	Cervical	1	3	
		30	1	Inguinal	1	3	
		35	8 hrs	Axillary	1	3	
-	+++	555	2	Nil	6	8	
			4		1	5	

TABLE F—*concld*

Bacteriological Diagnosis		Case	Duration of disease—days	Site of bubo	Number of treatments at intervals of 24 hours	Results	
Bubo	Blood					Death in days from the onset of the disease	Recovery
			3	*Nil*	1	3 (Pneumonic)	
+	++		[illegible]	Inguinal	1	4	
		9	5	Femoral	1	6	
		11	1		1	2	
		23	2		1	6	
−	++		2	*Nil*	1	5	
		10	[illegible]	"	1	7	

ANALYSIS OF CONTROL CASES

Series I

Moderate and severe septicæmic cases

Bacteriological Diagnosis		Case	Duration of disease—days	Site of bubo	Results	
Bubo	Blood				Death in days from the onset of the disease	Recovery
+	+++	558	4	Inguinal	7	
		561	4	Cervical	5	
		188	3	Femoral	5	
		189	3		4	
		2	4	Cervical	4	
		[illegible]	3	Inguinal	4	
		31	3	"	4	
		[illegible]	3	"	4	
		36	3	Cervical	3	
		40	5	Inguinal	5	
		44	2		2	–
		49	1	Femoral	3	

ANALYSIS OF CONTROL CASES—*concld*

Bacteriological Diagnosis		Case	Duration of disease—days	Site of bubo	Results	
Bubo	Blood				Death in days from the onset of the disease	Recovery
		53	1	Axillary	2	
		55	2	Femoral	3	
		57	2	Inguinal	2	
		64	2		3	
		81	4	Femoral	8	
		85	3		4	
		89	2	*Inguinal*	4	
		91	1	Femoral	2	
		92	3	Inguinal	3	
		101	3	Axillary	3	
		105	1	Femoral	2	
		115	3		3	
		121	1	Inguinal	2	
		130	1	Femoral	2	
		34	4	Axillary	6	
		39	3	Inguinal	5	
		40	2	Axillary	3	
—	+++	75	2	Nil	4	
		94	3		4	
		127	3		4	(Pneumonic)
		19	1		2	
		29	1		3	
		32	5		6	
+	++	20	3	Femoral	4	
		71	3	Inguinal	5	
		67	7		9	
—	++	103	1	Nil	4	
		8	2		2	
		16	1		2	

ANALYSIS OF CASES TREATED WITH BACTERIOPHAGE

Series II and III

Slightly septicæmic purely bubonic and clinically plague cases

Bacteriological Diagnosis		Case	Duration of disease—days	Site of bubo	Number of treatments at intervals of 24 hours	Results	
Bubo	Blood					Death in days from the onset of the disease	Recovery
+	+	560	5	Axillary	2	8	
		187	3	Femoral	1		R
			1	Axillary	1	8	
			4	Inguinal	1	4	
			2	Femoral	1		R
			1		1		R
			1	Inguinal	3	10 (Sec Pn)	
			3	Axillary	1	16 (Sec Pn)	
			3		2	7	
			6 hrs	Femoral	3	6	
		12	3	Inguinal	2	6	
		25	3	Femoral	1	8	
		33	2	Axillary	1	8	
		36	1		2	15 (11 day pustular rash)	
−	+		3	*Nil*	1	4 (Pneumonic)	
		5	2		1	4	
		7	4		1	5	
		15	1		1	2	
+	−	560	2	Inguinal	4	6	
		Tulja	5		2	9	
		Tulsa	3	Axillary	2		R
		176	10	Cervical	2		R
		180	3	Inguinal	1		R.
		184	5	Axillary	1	6	
			1		5		R
			4	Inguinal	1		R.

ANALYSIS OF CASES TREATED WITH BACTERIOPHAGE—*contd*

Bacteriological Diagnosis		Case	Duration of disease—days	Site of bubo	Number of treatments at intervals of 24 hours	Results	
Bubo	Blood					Death in days from the onset of the disease	Recovery
			3	Inguinal	2	7	
			3	„	2		R
			4	Axillary	1		R
			4	Inguinal	1		R
			2	„	2		R.
			2	„	2		R
			3	Inguinal	1		R
			2	„	1		R
			4	„	1	6	
			3	„	2		R
			5	Axillary	1		R
			4	Inguinal	1		R
			4	Cervical	1		R.
			3	Inguinal	3	11	
			3	Cervical	1		R
			3	Deltoid	1		R
			2	Femoral	1		R
			1	Axillary	1		R
			2	Femoral	1	4	
			2	Inguinal	2	4	
			1	„	1		R
			2	Deltoid	1		R
			1	Inguinal	1		R
			3		1		R
			3	„	1		R
			2	Inguinal and Cervical	1	5	
			3	Inguinal	1	3	

ANALYSIS OF CASES TREATED WITH BACTERIOPHAGE—*concld*

Bacteriological Diagnosis		Case	Duration of disease—days	Site of bubo	Number of treatments at intervals of 24 hours	Results	
Bubo	Blood					Death in days from the onset of the disease	Recovery
			4	Axillary and Cervical.	1		R
			1	Inguinal	1		R
		27	1	„	2	4	
		38	2	„	1	3	
		17	5	Femoral	1		R
—	—	570	4	Axillary	2		R
		Ramana	4	Inguinal	2		I
		Laxmi	2	*Nil*	1		R
			3	Femoral	2		R
			2	Axillary	1		R
			2	*Nil*	1		R.
			1	„	1	1	

ANALYSIS OF CONTROL CASES

Series II and III.

Slightly septicæmic, purely bubonic, and clinically plague cases

Bacteriological Diagnosis		Case	Duration of disease—days	Site of bubo	Results	
Bubo	Blood				Death in days from the onset of the disease	Recovery
+	+	170	3	Cervical		R.
+	+	34	4	Femoral	9	
		51	1	Axillary	5	
		77	4	Femoral		R.
		123	5	„	13	..
		118	3	Inguinal	7	..
		106	3	Cervical		R.

ANALYSIS OF CONTROL CASES—*contd*

Bacteriological Diagnosis		Case	Duration of disease—days	Site of bubo	Results	
Bubo	Blood				Death in days from the onset of the disease	Recovery
		6	3	Inguinal	7	
		24	4	Femoral	8	
—	+	109	3	Axl		R.
		4	2	„	7	
+	—	557	3	Inguinal		R
		572	?	„	4+?	
		186	3	Axillary		R
		8	5	Inguinal		R
		10	3	Cervical		R
		12	4	Axillary		R
		15	7	Inguinal		R
		18	2			R
		23	5	Femoral		R
		27	2	Inguinal	6	
		17	3	Cervical		R
		61	5	Inguinal		R
		66	2	,		R
		69	1	„		R.
		73	2	Femoral		R
		79	2	,	6	
		83	2	„		R
		97	1	Inguinal		R
		99		,	5	
		111	3	,		R
		113	3	Femoral		R
		125	3			R
		132	2	Supratrochlear		R
		2	2	Inguinal		R

ANALYSIS OF CONTROL CASES—*concld*

Bacteriological Diagnosis		Case	Duration of disease—days	Site of bubo	Results	
Bubo	Blood				Death in days from the onset of the disease	Recovery
		14	5	Inguinal		R
		20	3	"	7	
		22	2	"	8	.
		28	2			R
		37	4	"		R
—	—	55[illegible]	5	Nil	6	
		575	1		7	
		182	8		1[illegible]	
		Lamaya	1		2	
		4	2		6	
		6	2		8	
		30	2	Cervical		R
		38	3	Nil	Ran away from hospital	
		42	3		6	
		51	1		5	
		6[illegible]	2	"		R
		87	1	"		R
		117	2	"		R
		110	5	"		R
		13	4	"		R
		26	2	Inguinal		R
		31	3	Nil	4	

TABLE I

SUMMARIZED RESULTS OF TREATMENT OF HUMAN PLAGUE IN INDIA
1908—1926

Bacteriological Diagnosis Blood	Method of Treatment	Treated Cases		Control Cases		Remarks
		Number	Deaths	Number	Deaths	
+++	Serum	81	81	100	100	
	Iodine	14	14	8	8	Severe septicæmic cases
	Serum and Vaccine	12	12	5	5	
	Bacteriophage	32	32	35	35	
		139	139	148	148	Total
++	Serum	18	18	24	24	Moderately septicæmic cases
	Iodine	4	4	3	3	
	Serum and Vaccine	4	4	2	2	
	Bacteriophage	6	6	6	6	
		171	171	183	183	Total of both severe and moderate cases
+	Serum	49	3	35	20	
	Iodine	6	4	2	1	Slightly septicæmic cases
	Serum and Vaccine	6	5	1	1	
	Bacteriophage	18	15	11	7	
		79	59	49	29	Total

TABLE F—*concld*

Bacteriological Diagnosis.	Method of Treatment	Treated Cases		Control Cases		Remarks
Blood		Number	Deaths	Number	Deaths	
—	Serum	90	24	78	24	Clinically plague cases
	Iodine	16	7	7	3	
	Serum and Vaccine	18	6	12	5	
	Bacteriophage	47	13	45	15	
		250	109 (43 6 per cent)	191	76 (39 7 per cent)	Total of slightly septicæmic and clinical cases
	TOTAL OF ALL CASES	421	280 (66 5 per cent)	374	259 (69 2 per cent)	

SPECIFIC TREATMENT OF PLAGUE BY MEANS OF SERA AND VACCINES

BY

P T PATEL, M D, M R C P, D T M & H

Serum

C Bifulco (1926) quotes Montefusco's statement that the favourable *results obtained by various observers with anti plague serum were undoubtedly* due to the mildness of the prevailing epidemic rather than to the efficacy of the serum its methods of preparation, or the route by which it was injected In two epidemics of plague in 1904 and 1921 the mortality among the patients at the Contugno Hospital Naples, not treated with serum was only 11 5 per cent—a figure which is much lower than that obtained in India with anti plague serum either by the German mission (50 per cent) or by the Russian mission (40 per cent) Wigura and Jassenki had a mortality of 80 per cent among their cases of plague treated by serum at Bombay The failure of anti plague serum has been attributed by Terni to deficiency of bactericidal action and almost complete absence of plague anti toxin According to Bifulco, Montefusco is to be credited with having introduced an anti plague vaccine which he has employed in very severe *cases with successful results A daily dose of 5 c cs of the vaccine is given sub* cutaneously as long as there is no improvement or fall of temperature In many cases a considerable fall of temperature and improvement in the general condition occur after the first injection Treatment of plague by intra bubonic injection of D'Herelle's bacteriophage has only been given to patients who would probably have recovered without this treatment

Sensitized Vaccines

Major Stocker claimed some good results from a vaccine prepared by him and so we tried it in the Maratha Plague Hospital on about a dozen cases The following table shows that the results have been very unsatisfactory —

Number of cases treated with Sensitized vaccine 1 c c dose	Discharged cured	Died	Mortality rate per cent
12	2	10	83 3

In Bombay epidemics serum treatment has not given favourable results All observers are agreed that serum either Lustig s or from the Pasteur or Lister Institutes if given early in non septicæmic cases modify the disease in the direction of lengthening life but has produced only a small reduction (from seven to ten per cent) in the mortality rate A careful test carried out in Bombay by Liston in 222 cases with an equal number of controls showed a reduction of ten per cent in the mortality Choksy gave large doses of 100 c cs subcutaneously to about 500 cases and showed a reduction of ten per cent in the mortality Our statistics for serum treatment for the last five years are shown in the following table —

Method of treatment	Total cases	Discharged	Died	Mortality per cent
Sol Iodine (Alcohol) I V and anti plague serum (Pasteur) subcutaneous 40 to 60 c cs daily	63	16	47	74 60
Anti plague serum (Pasteur) subcutaneous and I V 20 to 80 c cs	100	29	71	71 00
Anti plague serum I V only 40 to 88 c cs (Pasteur)	65	17	48	74 00
Anti plague serum I V only 60 to 80 c cs (Lister Institute)	9	2	7	77 77

The above results are very contradictory The question as to virulence of various outbreaks the date of admission the date on which the treatment is given the presence or absence of septicæmia and the resistance of various individuals and races undoubtedly arise Thus for ascertaining statistically the value of a treatment in epidemic diseases we see that many variable factors are present It is necessary to have controls in the same outbreaks as the experiment and in the absence of comparative deductions in various epidemics it is valueless and misleading to draw definite conclusions as to the given benefit and reduction in the mortality Still from our experience of the serum treatment in this and other diseases it would not be justifiable to withhold it if freshly prepared serum is available

The method of treatment by serum in my opinion should follow the lines laid down from our experience with diphtheria anti toxic serum viz give quickly and in sufficient dosage in urgent cases by the intravenous route Repeat it as required by the evidence of the toxæmia and do not be afraid of it As in diphtheria do not allow the immediate beneficial effect of the neutralization of the toxins to overlook or under rate the serious damage already effected upon internal organs particularly the heart Do not allow too hurried convalescence In my experience the specific anti toxic serum in plague is as necessary a part of the treatment as is diphtheria anti toxin in diphtheria Right methods of use are required in each to get the best results A practical point—the desiccated serum (Pasteur) is equally efficacious

and keeps indefinitely No contra indication nor harmful sequelæ to serum treatment have been discovered Of course such treatment is not possible in the majority of cases here, because the cases come very late and also the price of the serum to carry out complete treatment is prohibitive, i e , something like Rs 100 per patient Further, protein shock -reactions and the disturbance to the colloid mechanism when a large amount of foreign protein is injected into the blood complicates the matter, so at present the question of efficacy of sera supplied now for the treatment of plague is *sub judice* All are agreed still that serum treatment may be of use with other treatment if given during the first 48 hours

Bacteriophage

D'Herelle reports four cases of bubonic plague treated exclusively by injection of a bacteriophage into the buboes To 10 c cs of a bouillon culture of the plague bacillus was added 3 c cs of fresh bouillon inoculated with a bacteriophage culture A dose of 1 c c of the filtrate was injected in the bubo, or two injections each of 0 5 c c, were given in two buboes In three patients injected the first day after appearance of a bubo, the general condition improved within a few hours after the injection Two injections were needed in a case in which the treatment was not started until the third day of the disease All were grave cases and all *rapidly recovered No other treatment was applied, but the bacteriophage* was known to be exceptionally active D Herelle suggests injecting 1 or 2 c cs into the buboes in dubious cases of plague, since it is absolutely harmless

In septicæmia and pneumonic plague, the injection should be made intravenously We tried the bacteriophage in some cases in the Maratha Plague Hospital but the results were not satisfactory, all cases proving fatal In another epidemic near Delhi the results were also the same, so it can be considered still on its trial

The Antiseptic Treatment

In the absence of perfect sera to destroy the bacilli and neutralize their toxins, various attempts are made to achieve this by means of antiseptics Large doses of carbolic acid, perchloride of mercury and various preparations of iodine and chlorine from time to time have been put forth as curing numbers of cases but it is difficult to understand how much antiseptics can help when there is such an amount of overwhelming toxæmia from the beginning, if the toxæmia can be combated either by anti toxins or by the resisting powers of the patient, the remaining slight infection may possibly be destroyed by potent internal antiseptics as iodine or chlorine which at the same time do not injure the body cells Vassalo reports a series of plague cases in Uganda showing good results with treatment by iodine He used a *freshly prepared solution of—*

Iodine	1 drachm
Potassium Iodide	1 oz
Absolute Alcohol	20 ozs ,

10 to 15 minims with 10 c cs of saline to be injected intravenously once daily

On looking over the reports of the treatment of plague by various chemicals since 1896, I find that various observers have tried preparations of iodine and carbolic acid in a haphazard way in a certain number of cases varying from 50 to 100

Iodine preparations such as tinctures, aqueous solutions and colloidal solutions varying from 5 to 20 minims were given either by mouth or intravenously and the mortality has always been from 65 to 75 per cent hardly less than in the cases without any treatment

Carbolic acid and Izal have also been given in heroic doses totalling ½ to 2 drachms by mouth during 24 hours but have not produced any effect on the course or temperature of the disease on the other hand they have produced marked hæmoglobinuria in some cases For this purpose we carried out a series of observations with various suggested chemicals in a number of definite and accurate plague cases, so as to come to definite conclusions as to their value in treatment The following tables show the number of plague cases treated with iodine* and its preparations and other antiseptics —

IODINE

Year	Cases treated	Recovered	Died	Mortality per cent
1922 23 24 25	409	97	312	73 0

MERCURIC PREPARATIONS

Sol Mercurochrome ½ to 2 per cent, 5 to 10 c cs injected intravenously

Year	Cases treated	Recovered.	Died	Mortality per cent
1925	6		6	100

* *Tinct Iod or Liq Iodi (Alcoholic)*

Iodine	1½ drachms
Potassium Iodide	1 oz
Absolute Alcohol	20 ozs

Dose =10 to 15 minims with aqua dist 1 10 c cs injected intravenously and subcutaneously near the site of bubo once daily

Aqueous solutions of Iodine

Potassium Iodide	36 grs
Iodine	24 grs.
Aqua Distil	1 oz.

Dose =1 to 2 c cs injected intravenously

Liqr Iod. Terchlor by mouth in 7 to 20 minims doses in one ounce of water thrice daily

RESOLUTIONS PASSED AT THE JOINT MEETING OF THE EXPERT PLAGUE COMMITTEE OF THE LEAGUE OF NATIONS HEALTH ORGANIZATION, AND THE F E A T M

The following investigations are considered of particular importance —

(A) BUBONIC PLAGUE

(1) Further investigations into the methods of destruction of rats and fleas

(2) Investigation on the comparative epidemiological rôle of the various species of fleas in plague transmission in selected areas of India as being the most heavily infected country the species of fleas concerned and their viability under natural conditions

(3) Survey of plague in wild rodents of Northern Asia (Transbaikalia Manchuria and other Chinese Provinces) by an international mission provided such mission receives substantial support from the countries concerned

(4) Investigation on the part played by grain and cotton in the dissemination of plague and measures to prevent this spread (disinfestation)

(5) Investigation of the conditions under which plague is carried over from one season of incidence to another (problem of its recrudescence)

(6) Investigation on the relative importance of rodents other than rats in the transmission of plague in various countries

(7) Investigation of rat and flea conditions in ports (shore lighters ships) the ship fauna being investigated both in ports and during the voyages in eastern and western areas This information should be collected by the Singapore Bureau for providing information applicable to quarantine measures

(8) Prophylaxis and therapeutics

(*a*) Speedy preparation of anti plague vaccine

(*b*) Possibility of reducing local reaction to anti plague vaccine

(*c*) Possibility of producing a plague anti toxic serum

(*d*) Further studies on anti plague bacteriophage and its practical applications

(*e*) Chemo therapy of plague

(B) PNEUMONIC PLAGUE

(1) Investigation of the incidence of bubonic plague cases in outbreaks of pneumonic plague relative incidences of cases of bubonic plague secondary pulmonary plague and primary pneumonic plague in the various outbreaks

(2) Study of the possibility of existence of a special ultra virus or filter passing form of *B pestis* as the causative agent of pneumonic plague

CHOLERA

STATISTICAL STUDIES IN THE EPIDEMIOLOGY OF CHOLERA

BY

LIEUT COL A J H RUSSELL, C B E, M A, M D, D P H, I M S,

Director of Public Health Madras

INTRODUCTION

TUESDAY, DEC. 6TH, 10 A.M. TO 1 P.M.

ONE of the most serious problems which Public Health Officers in India are called upon to face is the control of the recurring and extensive outbreaks of epidemic cholera

The bulletins issued by the League of Nations indicate that at the present time India is practically the only part of the world in which cholera persists in endemic form and as Bengal has been called the 'home of cholera' by most writers during the past 200 years it seems fitting that this disease should form the subject of discussion at an International Congress held in Calcutta, the heart of that province and the first city of the Indian Empire

A review of historical records makes it evident that the disease known as cholera was familiar to the Hindu Chinese Arab Greek and Roman writers of the pre Christian era and that in India 'the cholera of to day is exactly the same as it was at least 400 years ago and as it probably ever has been (2) Because of India's position as the source of infection to other countries it has been the unfortunate custom in discussing the epidemiology of cholera, to look upon the epidemics there as relatively unimportant and to devote most time and energy to tracing the routes of spread from India to other parts of the world It is obvious that the epidemiology of cholera as it exists in India is the key to the problem and it is surprising how little attention the epidemiological features of the disease have received in the past, most writers having been content either to ignore the question altogether or make vain repetition of previously recorded inaccuracies If the causal factors influencing the periodic outbursts of the disease in this country could

be elucidated and combated its spread to other countries would cease altogether or in any case cease to be of any importance

Available Statistics

We have in India extensive records from 1866 onwards dealing with the incidence of the main epidemic diseases, including cholera Whilst these afford 'a rich field for epidemiological studies of a tropical climate which has not hitherto been adequately explored' (15) it must be admitted that they relate almost entirely to the more violent outbursts of infectious disease, and, because of defective registration very frequently fail to indicate the large number of smaller epidemics The percentage of error however is by no means so great as to render the cholera statistics valueless and, moreover if the available monthly figures are taken over a sufficiently long period of time, a fairly accurate representation of the varying incidence of the disease can be obtained

Cholera in India is a very familiar and easily recognized disease, and, although many cases of diarrhœa are no doubt wrongly included it has been found that results are not vitiated to any extent by assuming that all deaths registered as cholera were actually such By using the statistics for different provinces it has been possible for example to forecast epidemics of cholera two or three months ahead of the actual outbreaks(7) It may therefore be stated with confidence that the available data are sufficiently accurate for purposes of comparative epidemiological study and that they permit of definite inferences being made

Endemic and Epidemic Areas

A study of the annual cholera deaths over a long period of years has made it possible(7) to divide the provinces of India into three great groups —

I The first group includes the provinces of Assam Bengal, Bihar and Orissa and the United Provinces where more or less uniform figures are registered annually and where the average incidence is high These areas are very likely to be endemic in nature

II In the second group are included the Central Provinces Bombay Presidency and the Punjab and North West Frontier Province where sudden peaks in cholera incidence occur at irregular intervals These areas are normally free from cholera epidemics and infection is probably always brought in from outside

III The Northern and Central Districts Groups of Madras Presidency are epidemic areas whilst the Southern Districts Group which presents a more uniform incidence might almost be included in Group I as an endemic area

This differentiation of the statistical areas of India into epidemic and endemic groups has been amply verified by a number of independent methods applied in connection with the forecasting of cholera epidemics(7) The epidemic indices,

the monthly mean and median deaths, and the zero order and partial correlation coefficients for each area have all corroborated this classification

That cholera tends to recur repeatedly in river deltaic tracts, especially in localities inundated by periodical floods, is a well known fact, and it is interesting to note that the main endemic areas of India include, and lie around, the deltaic tracts of the Ganges, Brahmaputra, Cauvery and other large rivers Epidemic records show that, again and again outbreaks have commenced in the towns and villages lying on river banks and that infection rapidly and systematically spreads down these rivers Moreover, there is no question that, in endemic areas, cholera spontaneously appears, year after year, in the same villages and towns In other areas, *per contra* it is necessary for other favourable conditions to be present before cholera becomes diffused, e g overcrowded and insanitary conditions associated with religious fairs and festivals

PERIODICITY OF CHOLERA EPIDEMICS

As with other epidemic diseases, cholera spreads widely and rapidly at certain periods, whilst at other times, it remains dormant or spreads only sporadically and with difficulty The regular seasonal appearance of the disease in more or less virulent form is a well recognized characteristic of its manifestations but in certain areas for example those of South India this form of periodicity becomes apparent only when due consideration is given to varying geographical features Periodicities of a longer duration while not obvious have been demonstrated by the application of the periodogram method used by Brownlee(2 and 25) By this means, it has been found that, in nearly all the areas where cholera is epidemic waves of the disease recur once every five to six years whilst in the endemic areas, a 4 5 years periodicity is most probable In every case the periodograms show that cholera tends to run a more or less definite course of revival, decline and subsidence in each cycle of years This phenomenon has been demonstrated further by the epidemic indices curves relating to the different areas of India(7)

It must not be understood, however that cholera in India adheres to a regular cycle As Sir Leonard Rogers(11) has stated the problems associated with the epidemiology of cholera are not so simple as to be explained by a cyclic trend Koch attempted to explain its periodicity 'mainly through the influence of the immunity which follows extensive ravages of the disease' Probably other factors have equal significance, but whatever influences may be at work it is certain that fore knowledge of the probable advent of a periodic peril in the incidence of the *disease would go far to prevent waste of effort in unnecessary directions and at* unnecessary seasons In Madras, we have for three years past made use of that knowledge with very considerable success'

CLIMATE AND CHOLERA

Nearly all the earlier medical writers emphasize the close relationship between climate and health Bellew(3) expressed the opinion that the great difference of

the prevalence of cholera in Europe and America and in India is the striking regularity of the periodic recurrence in epidemic form in India' 'This' he says 'is only confirmatory of the view that the disease depends for its origin as well as its epidemic development upon influences of weather for in no other country is the succession of seasons marked by meteorological phenomena of such magnitude and violence or by such sudden and great changes in the conditions and states of the weather elements and with also such regularly recurring periodicity as they are in India'

It is obvious that the true effects of climate on health are more easily traceable in primitive communities In other areas the effects of 'sanitation food water habit altitude character and moisture of the soil race traffic and other controls serve to complicate the problem'(20)

'The cause of disease is no longer sought directly in meteorological conditions but in the effect more or less direct of these conditions upon the micro organisms which are the specific cause of the disease Atmospheric conditions may help or may retard the development of the micro organism and may strengthen or weaken the individual's power of resistance against the attacks of the germ (20)

Perhaps the most painstaking attempt to estimate the influence of climatic factors on cholera incidence was undertaken in 1916 in the Philippine Islands by a group of American workers(3) Their conclusion was that while there are apparently some related factors to be seen so far they are so elusive that nothing definite can yet be stated' As most of those who had devoted any attention to the subject were agreed that weather conditions in some way exert a considerable influence it *seemed worth while to undertake the detailed statistical analyses of the available* figures for India which have been published by us during the last three years

Climatic Conditions

In any study of the influence of weather on the prevalence of disease the assignment of increased mortality to any particular factor is not nearly so simple as is generally believed (20) Climate is an extremely complex subject and all that *can be done is to attempt to measure the degree of association of the various* elements included in the term climate with the incidence of disease The influence of climate is in fact determined not by temperature alone nor by humidity alone nor by rainfall alone but by combinations of all three

Temperature has an important influence on plant and animal life and on the life and occupation of man but the distribution of plant and animal life and the [illegible]

to any direct evil effects of damp on the human system as to the fact that the

agents and carriers of disease find in heat and moisture the conditions that best favour their growth and multiplication '(19) Rain has been supposed to exert a direct influence on the distribution of disease, but it is most probable that its precipitation acts only indirectly 'A prolonged drizzle in a warm climate simply turns the soil into a particularly efficient cultivation ground for the germs of infective disease, and the attendant gloom of the sky stops entirely the beneficent germ killing power of the sun's direct rays '(19)

'From a sanitary point of view, the variations of the barometer are of little interest, as at any given level they are never sufficiently great to have physiological effect on the human organism '(19) The meteorological records for pressure being available however, this factor was also taken into consideration, although it was not thought likely that it could have any very appreciable association with the incidence of cholera

We have it from Sir Leonard Rogers that 'reference to the climatic data shows at once no relationship between seasonal cholera incidence and either rainfall mean temperature or relative humidity, but when we turn to the absolute humidity data we find the clue to the problem '(14) The first half of this statement is definitely misleading, as will be shown later The arguments brought forward in favour of an absolute humidity figure of 0 40 seem also to be based on broad generalizations With all due deference, it is suggested that conclusions of this kind cannot possibly be reached without submitting the available data to detailed statistical analyses and it does not appear that such methods were employed

Moreover, 'dampness and dryness, which depend quite as much on the temperature as on the quantity of vapour present in the air, are the conditions most important to us, both in respect of our own bodies and also as affecting vegetation of all kinds In technical language, this is spoken of as the relative humidity of the air, in contra distinction to the absolute humidity, which has reference only to the amount of water vapour present independently of the temperature '(26) In view of these facts, it is clear that the clue to the cholera problem is not to be found in absolute humidity or in any other individual climatic factor

Simple and Partial Coefficients of Correlation between Cholera Incidence and Climatic Factors

In most epidemics a large number of factors come into play and, in any statistical analysis as many of these as possible should be taken into consideration In actual practice, however, certain limitations exist In our studies we have tried to measure mathematically the relation between the incidence of cholera and rainfall, humidity, temperature and pressure other factors having been ignored for the time being

The monthly averages given in the official reports in respect of each of these four factors were collected for all meteorological stations lying within the thirteen areas into which India was divided for statistical purposes From these, average

monthly figures for each area were calculated(8) The monthly cholera deaths showed occasional extreme fluctuations either on account of severe outbursts of the disease or delayed registration The figures were, therefore, smoothed out by transforming them into moving deviations or deviations from a moving average(3) Apparently no such graduation which seems to be an essential preliminary to any scientific examination of the figures was adopted by Sir Leonard Rogers and the rates he used were therefore frequently misleading

The monthly figures for cholera and each climatic factor in turn were set up in correlation tables(8) The zero order coefficients of correlation so obtained were then used for the *determination of partial coefficients of all orders (5 and 6) (see* Table at the end of text) As far as cholera epidemics are concerned it is of little advantage to try to locate the part played by any one climatic factor when the others are held constant From such an analysis however, useful and definite inferences can be drawn in estimating the role of the individual factors in the sum total of their combined influence on the incidence of cholera For this purpose multiple correlations were also computed and as all of them were significant they gave some measurable justification for the general belief that climatic factors have a considerable influence on the incidence of cholera

In the case of the zero order correlations coefficients for lags of one and two months were computed for each weather factor in each area(3 and 4) Not only were the lag coefficients for temperature and cholera significant—in some instances indeed they were as high as 0 5—but the values for lag were considerably higher than those for lag$_0$ From this it would appear that although temperature is definitely associated with the incidence of cholera the maximum effect is obtained only after a period of about one month

INTERPRETATION OF THE CORRELATION COEFFICIENTS

It is obvious that in a free atmosphere a *condition in which all factors but one* are held constant is impossible to reach As our object was to estimate the role of each climatic factor in the more or less inconstant average atmosphere over a large area it was important to consider the influence of each individual factor on the associations of the other *factors with cholera in a changeable* atmosphere

Interpretations of the large number of coefficients of correlation with special reference to the varying conditions obtaining in different areas are given in detail in a final paper on the subject(6) which appeared recently as a *Memoir* of the *Indian Journal of Medical Research*

After making due allowance for differences in the relationships of climatic factors and in the physical features of the areas considered the following groups of coefficients for the different areas seem to us to give adequate material for the purpose in view

A	12 —0·0882	B_4	12 35 —0·0174	Pr	12 35 +0·1337
	13 —0·1182		13 25 —0·3028		12 354+0·1207
	14 —0·0242		14 35 —0·0851		13 25 +0·0207
	15 —0·1010		14 352—0·0877		13 254+0·0518
			15 24 +0·0108		14 235+0·0746
			15 243—0·0108		15 234—0·0581
B_1	12 35 —0·0935	B & O	12 35 —0·0360	By	12·345+0·1144
	13 25 +0·0371		13 354—0·0611		13 245+0·0401
	13 254+0·0109		13 25 +0·0970		14 235+0·0692
	14 35 +0·3104		13 254+0·1473		15 234—0·0894
	15 24 +0·2870		14 35 +0·1576	M_1	12 35 —0·0367
			15 24 —0·1696		13 25 +0·1887
			15 243—0·1962		14 235—0·0681
					15 23 —0·3158
B_2	12 35 —0·0424	U P	12 345—0·1688	M_2	12 345—0·1811
	13 25 —0·2423		13 245+0·2649		13 245+0·1388
	14 35 —0·0102		14 235+0·3168		14 35 —0·3599
	14 352—0·0211		15 24 —0·0074		15 234—0·1306
	15 24 +0·1989		15 243—0·0032		
	15 243+0·1806				
B_3	12 35 —0·0366	C P	12·345+0·1063	M_3	12 345—0·1662
	13 25 —0·1733		13 254+0·0013		13 245+0·0661
	14 35 +0·0903		13 25 —0·0366		14 35 —0·3409
	14 352+0·0268		14 352+0·0859		15 34 —0·1340
	15 24 +0·1849		14 35 +0·0845		
	15 243+0·1888		15 24 +0·0689		

From these series, the following inferences can be made —

(1) Some parts of India are endemic with respect to cholera that is, in those areas, the climatic factors appear to have no influence whatever on the incidence or spread of epidemics This group includes Assam, Bengal and the southern and central areas of Madras Presidency The endemic characteristics of the Madras areas are, however, different from those of Bengal and Assam Whilst in

the latter, cholera normally subsides in the season of high humidity, in the *former only when high humidity* prevails does the disease assume an epidemic form

(2) In contra-distinction of the endemic areas, other parts of India appear to suffer from cholera in epidemic form only In these epidemic areas, which include Bihar and Orissa, the United Provinces and the northern districts group of Madras Presidency, rainfall has *either a negative or an insignificant correlation with* cholera

(3) In addition to these two groups, certain Provinces may be said to be neither endemic nor epidemic, for the reason that they only occasionally suffer from cholera and then usually in the rainy season only, and when infection is imported from outside This group includes the Punjab and North West Frontier Province, Bombay Presidency and the Central Provinces

In a brief paper of this kind it is impossible to present, in any suitable *abbreviated form, the large number of correlation* coefficients which were computed and taken into consideration, but the degree of significance of the climatic factors in each area is indicated in the following statement, by plus and minus signs(6)

	Areas	R	H	T	P
	A		—		—
	B_1			+++	++
I Endemic areas	B_2		— —		+
	B_3		—		+
	B_4		— — —		
	M_2	—	+	— — —	—
	M_3	—		— — —	—
	B & O		+	+	—
II Epidemic areas	U P	—	++	+++	
	M_4		+		— — —
	C P	+			
III Free areas	Pr	+			
	By	+			

The signs in all chambers of the table for M_2 and M_3 areas in Group I are the exact opposite of those in the other endemic areas of that group The differentiation between the two is very striking In the third group, rainfall

alone plays any part but this weather factor has no significance other than a negative one either in the endemic or epidemic areas Rainfall therefore does not have any direct effect on the incidence of cholera but merely assists in the distribution of infection

The other climatic factors are active rather than passive and are of importance in determining the virulence of epidemics In epidemic areas the combination of high relative humidity with high temperature accompanied by intermittent rainfall constitutes a favourable atmosphere for the spread of cholera In endemic areas however, such a combination is not necessary either for its development or spread

This conclusion can be verified by a detailed study of the variations of temperature and relative humidity in different parts of India In M_1 B_2, C P B & O and U P, high temperature and high humidity coincide with intermittent rainfall in the rainy season During the same months Bengal and Assam have heavy rainfall with high temperature and humidity but M and M_3 and P have high temperature with practically no rain and only moderate humidity During this season cholera is absent from the latter areas as well as Bengal and Assam but occurs in other parts of India

In the south east of Madras Presidency temperature falls suddenly with the burst of the north east monsoon but the daily variation is small and humidity is very high These facts explain the lag of from one to two months which has been demonstrated, for it is only when temperature is re established in a high humidity atmosphere that, in this part of India cholera incidence reaches its peak

There does not seem to be much doubt that a close connection exists between the endemic centres and the development of epidemics in other areas In the endemic areas, epidemics periodically spring into existence fresh outbursts regularly following quiescent periods It is now clear that these epidemic outbursts are mere intensifications of the endemic disease, influenced partly by favourable humidity and temperature conditions, but probably also by other conditions not precisely known Here, no doubt, the chronic carrier plays his part The existence of the cholera carrier has been conclusively demonstrated by Greig and other workers, and the regular outbreaks of cholera, which originate during or immediately after religious fairs and festivals, can only be explained by the presence among the pilgrims of numbers of 'carriers' of the cholera bacillus, the conditions at these fairs and festivals stimulating to activity the latent infection in those persons

Conclusion

Examination of the mortality data for India as a whole has shown that some provinces are endemic with respect to cholera, others are epidemic, and a few are more or less free(7) Seasonal and long wave periodicities of the incidence of cholera have been demonstrated(2 and 4) Finally, the mathematical evaluation of the association of the incidence of cholera with variations in the climatic factors

rainfall, relative humidity, temperature and pressure(3, 4 and 5), and the comparative study of the partial and zero order coefficients of correlation for all India have indicated that it is no longer mere theory to suppose that climatic factors have a definite relationship with the incidence of cholera in India, *although in dealing with disease phenomena, many and varied influences are at work* Local weather conditions, seasonal incidence, race distribution, sex, age, social conditions, poverty, etc , are some of the important factors involved, and in statistical analyses, it is possible to consider only a few of these Consideration of the climatic factors alone has demonstrated the important part played by humidity and temperature, but with Topley 'we must assume that during the pre epidemic phase some process goes forward which leads to a progressive alteration in the equilibrium between parasite and host, and that it is only when a certain limiting condition has been reached that an epidemic wave of mortality is propagated'(12) It is suggested that, in the case of cholera in India, this pre epidemic phase is likely to be determined by the association of high relative humidity with high temperature, accompanied by intermittent rains The presence of endemic centres, however, from which epidemics spring at short intervals, is a fact which must be accepted No single factor can be held responsible for the periodic waves of the disease which devastate the provinces of India, as these waves are preceded by conditions too complex to admit of complete solution with the help of available data Individual susceptibility, foci of infection, favourable atmospheric conditions, *fairs and festivals, carriers, insanitary habits, all play their part* '

The question, whether cholera can be extinguished in India, is, therefore, meantime premature, although at the same time eventual control of the disease may be considered certain ' Practical measures for the prevention of cholera can only be founded on the observation and recognition of the facts which the disease ordinarily presents If this principle be kept constantly in view, and mere theory be carefully avoided, we believe that very much may be accomplished '

The neglect of hygienic measures, although no doubt greatly influencing the spread of cholera, cannot by any means be considered its sole cause, because tracts of country sometimes escape where conditions are just as insanitary as those infected There can, however, be no doubt that, in spite of favourable climatic conditions, hygienic measures such as the protection of water supplies can, and do, prevent the development and spread of the disease This has been amply proved by the provision of protected supplies not only in the larger municipal towns but to some of the important religious festival areas

The question of population deserves consideration in relation to the control of epidemics in India, as public health activities must always be intimately bound up with the problem of population It has been shown separately(9) that India, as a whole, has almost reached saturation point under present conditions Few realize that India is a densely crowded country where each individual consciously or unconsciously is already challenging the right of every other individual to existence.

It is not the purpose of this paper to make dogmatic statements either on population or on the cholera question This much however, can be said that so long as public health departments confine their attention merely to the eradication of disease so long will their efforts end in disappointment

The proposal to protect millions of pilgrims year after year by means of the anti cholera vaccine is one which might make the boldest public health administrator submit his resignation Inoculation against cholera is no new experiment in India and public health authorities are of course well acquainted with the prophylactic value of the anti cholera vaccine

Compulsory methods might appeal to men accustomed to deal with disciplined troops or to those who plan preventive campaigns on paper but those with administrative experience will it is certain be unanimously of the opinion that compulsory mass inoculation is not the correct way to tackle the cholera problem in this country With few exceptions the people of India are still ignorant of the purpose and plan of public health activities and they are not only suspicious of new ideas but resent interference with established habit and custom

It is obvious in any case that inoculation by itself cannot be expected to eradicate cholera unless extensive sanitary arrangements are made at important towns and trading centres and at the multiple fairs and festival centres to be met with in all parts of India The provision of pure water supplies rapid collection and disposal of refuse and night soil the extension of health organizations and staffs the immediate notification of outbreaks of the disease are all important essentials which are receiving more and more attention from provincial Governments and Public Health Departments In the great task of controlling cholera in India, we need the co operation not merely of the Governments in India but of all interested in the welfare of this country The support of such international bodies as the League of Nations and the Far Eastern Association of Tropical Medicine will also go far to ensure advance

Whilst it is perhaps impossible to defeat the influence of favourable climatic factors, it ought not to be beyond the skill of man with all the weapons which modern science has placed at his command to devise measures to meet successfully many of the other influences at work and only when these are introduced and when public opinion in India demands their introduction will it be possible to hope for the control and eventual eradication of this deadly enemy of mankind

REFERENCES

(1) Russell, A J H (19 5) A Memorandum on The Epidemiology of Cholera published by the Health Section League of Nations C H 339 July

(2)* *Idem* (19 5) Epidemiology of Cholera (I) *Ind Jour Med Res* October

(3)* *Idem* (1926) Epidemiology of Cholera (II) *Ibid* January

(4)* *Idem* (1926) Epidemiology of Cholera (III) *Ibid* July

* See also *Ind an Medical Research Memoirs* No 1* October 19 5

(5)* RUSSELL, A J H with SUNDARARAJAN, E P (1926)	Epidemiology of Cholera (IV), *Ind Jour Med Res*, October
(6) *Idem*, (1928)	*Epidemiology of Cholera (V), Ind Med Res Memoir*, No 12, October
(7) *Idem*, (1927)	Forecasting of Cholera Epidemics, *Ind Jour Med Res*, April
(8) *Idem* (1927)	A short and simple method of construction and reduction of correlation tables, *Ibid*, January
(9) RUSSELL A J H (1927)	Population and Public Health in India' Transaction of the 7th Congress Far Eastern Association of Tropical Medicine, Vol I, p 903, December
(10) *Idem*	Cholera Bilivaccin and Anti cholera Vaccine (a comparative field test) Contribution to the Health Section of the League of Nations
(11) ROGERS, L (1905)	Letter on 'The Periodicity of Cholera in India,' *Lancet* June 20
(12) TOPLEY, W W C (1926)	Lecture on 'Experimental Epidemiology,' *Ibid*, March 6
(13) ROGERS, L	Incidence and spread of cholera Section of Epidemiology and State Medicine, Royal Society of Medicine.
(14) *Idem* (1927)	The Forecasting and Control of Cholera Epidemics in India, *Jour Roy Soc Arts*, February, 18
(15) *Idem*	'Smallpox and Climate in India,' Medical Research Council, Special Report Series No 106
(16) LOUIS, J and HARRIS, M D (1925)	Values in the control of communicable disease, *Amer Jour Public Health*, April
(17) LLOYD ARNOLD (1927)	The Auto Sterilizing Mechanism of the Gastro Intestinal Tract *Ind Med Gaz*, August
(18) FRY, A B (1925)	Cholera in Bengal Past and Present, *Ibid*, July
(19) GILES, G M (1904)	Climate and Health in Hot Countries'
(20) WARD ROBERT DECOURCY (1909)	Climate considered especially in relation to man'
(21) ROSS and BAGCHI (1918)	Seasonal variation in the reaction and hardness of river water in India *Ind Jour Med Res*, Vol VI
(22) *Idem* (1924)	Seasonal variation in the reaction and hardness of river water in India, *Ibid*, Vol XII
(23) TOMB, J W (1923)	A note on an investigation into the value of Essential Oils in the prevention and treatment of cholera, *Ind Med Gaz*, Vol LVIII
(24) JOLLY, G G (1926)	Cholera and River Waters, *Ibid*, April.
(25) BROWNLEE, JOHN	'Periodicities of Epidemics of Measles in the large towns of Great Britain and Ireland'
(26) BLANFORD H F (1899)	'The climates and weather of India Ceylon and Burma' (Based chiefly on the Publications of the Indian Meteorological Department)

* See also *Indian Medical Research Memoirs*, No 12, October 1928

TABLE

COEFFICIENTS OF CORRELATION OF ALL ORDERS FOR ALL INDIA

Key to Subscripts

CHOLERA (1)
RAINFALL (2)
HUMIDITY (3)
TEMPERATURE (4)
PRESSURE (5)

TABLE

A

	$r_{12.3} = -.0769$	$r_{12.34} = -.0787$	
		$r_{12.35} = +.0514$	
$r_{12} = -.0882$	$r_{12.4} = -.1165$	$r_{12.45} = -.0121$	$r_{12.345} = +.0313$
		$r_{12.43} = -.0787$	
	$r_{12.5} = +.0033$	$r_{12.53} = +.0514$	
		$r_{12.54} = -.0121$	
	$r_{13.2} = -.1101$	$r_{13.24} = -.1051$	
		$r_{13.25} = -.1355$	
$r_{13} = -.1182$	$r_{13.4} = -.1222$	$r_{13.45} = -.1076$	$r_{13.245} = -.1657$
		$r_{13.42} = -.1051$	
	$r_{13.5} = -.1256$	$r_{13.52} = -.1355$	
		$r_{13.54} = -.1076$	
	$r_{14.2} = +.0800$	$r_{14.23} = +.0728$	
		$r_{14.25} = +.1386$	
$r_{14} = -.0242$	$r_{14.3} = -.0397$	$r_{14.35} = +.1218$	$r_{14.235} = +.1687$
		$r_{14.32} = +.0728$	
		$r_{14.52} = +.1386$	
	$r_{14.5} = +.1380$	$r_{14.53} = +.1218$	
	$r_{15.2} = +.0495$	$r_{15.23} = +.0937$	
		$r_{15.24} = +.1237$	
$r_{15} = +.1010$	$r_{15.3} = +.1096$	$r_{15.34} = +.1586$	$r_{15.234} = +.1780$
		$r_{15.32} = +.0937$	
	$r_{15.4} = +.1690$	$r_{15.42} = +.1237$	
		$r_{15.43} = +.1586$	

TABLE—*contd*

B₁

r12 = −·2939	r12·3 = −·2493 r12·4 = −·3181 r12·5 = −·1385	r12·34 = −·2869 r12·35 = −·0935 r12·45 = −·1328 r12·43 = −·2869 r12·53 = −·0935 r12·54 = −·1328	r12·345 = −·1153
r13 = −·1613	r13·2 = −·0127 r13·4 = −·1513 r13·5 = −·1093	r13·24 = +·0484 r13·25 = −·0371 r13·45 = −·0669 r13·42 = +·0484 r13·52 = −·0371 r13·54 = −·0669	r13·245 = +·0109
r14 = −·0933	r14·2 = +·1574 r14·3 = −·0743 r14·5 = +·3[illegible]10	r14·23 = +·1640 r14·25 = +·3189 r14·35 = +·3104 r14·32 = +·1640 r14·52 = +·3189 r14·53 = +·3104	r14·235 = +·3171
r15 = +·2683	r15·2 = +·0[illegible]1[illegible] r15·3 = +·[illegible]4[illegible]0 r15·4 = +·4003	r15·23 = +·0704 r15·24 = +·[illegible]8[illegible]0 r15·34 = +·380[illegible] r15·32 = +·0[illegible]04 r15·42 = +·2870 r15·43 = +·380[illegible]	r15·234 = +·2[illegible]33

TABLE—*contd*

B₂

$r_{12} = -5753$	$r_{12\cdot3} = -4557$ $r_{12\cdot4} = -3942$ $r_{12\cdot5} = -1852$	$r_{12\cdot34} = -1922$ $r_{12\cdot35} = -0424$ $r_{12\cdot45} = -1553$ $r_{12\cdot43} = -1922$ $r_{12\cdot53} = -0424$ $r_{12\cdot54} = -1558$	$r_{12\cdot345} = -0458$
$r_{13} = -4198$	$r_{13\cdot2} = -1577$ $r_{13\cdot4} = -4225$ $r_{13\cdot5} = -2977$	$r_{13\cdot24} = -2531$ $r_{13\cdot25} = -2423$ $r_{13\cdot45} = -2780$ $r_{13\cdot42} = -2531$ $r_{13\cdot52} = -2423$ $r_{13\cdot54} = -2780$	$r_{13\cdot245} = -2392$
$r_{14} = -4977$	$r_{14\cdot2} = -2243$ $r_{14\cdot3} = -4997$ $r_{14\cdot5} = +1110$	$r_{14\cdot23} = -2975$ $r_{14\cdot25} = +0447$ $r_{14\cdot35} = -0102$ $r_{14\cdot32} = -2975$ $r_{14\cdot52} = +0447$ $r_{14\cdot53} = -0102$	$r_{14\cdot235} = -\cdot0211$
$r_{15} = +6057$	$r_{15\cdot2} = +2934$ $r_{15\cdot3} = +5473$ $r_{15\cdot4} = +4108$	$r_{15\cdot23} = +3433$ $r_{15\cdot24} = +1989$ $r_{15\cdot34} = +2579$ $r_{15\cdot32} = +3433$ $r_{15\cdot42} = +1989$ $r_{15\cdot43} = +2579$	$r_{15\cdot234} = +1806$

TABLE—*contd*

B_3

	$r_{12\cdot3} = -\cdot3691$	$r_{12\cdot34} = -\cdot1806$	
		$r_{12\cdot35} = -\cdot0366$	
$r_{12} = -\cdot4725$	$r_{12\cdot4} = -\cdot3$[illegible]77	$r_{12\cdot45} = -\cdot0874$	$r_{12\cdot345} = -\cdot0370$
		$r_{12\cdot43} = -\cdot1806$	
	$r_{12\cdot5} = -\cdot1388$	$r_{12\cdot53} = -\cdot0366$	
		$r_{12\cdot54} = -\cdot0874$	
	$r_{13\cdot2} = -\cdot1163$	$r_{13\cdot24} = -\cdot1571$	
		$r_{13\cdot25} = -\cdot1733$	
$r_{13} = -\cdot3360$	$r_{13\cdot4} = -\cdot3110$	$r_{13\cdot45} = -\cdot1862$	$r_{13\cdot245} = -\cdot1619$
		$r_{13\cdot42} = -\cdot1571$	
	$r_{13\cdot5} = -\cdot2178$	$r_{13\cdot52} = -\cdot1733$	
		$r_{13\cdot54} = -\cdot1862$	
	$r_{14\cdot2} = -\cdot0858$	$r_{14\cdot23} = -\cdot1383$	
		$r_{14\cdot25} = +\cdot0681$	
$r_{14} = -\cdot3740$	$r_{14\cdot3} = -\cdot3574$	$r_{14\cdot35} = +\cdot0263$	$r_{14\cdot235} = +\cdot0268$
		$r_{14\cdot32} = -\cdot1383$	
	$r_{14\cdot5} = +\cdot0836$	$r_{14\cdot52} = +\cdot0681$	
		$r_{14\cdot53} = +\cdot0263$	
	$r_{15\cdot2} = +\cdot1932$	$r_{15\cdot23} = +\cdot2312$	
		$r_{15\cdot24} = +\cdot1849$	
$r_{15} = +\cdot4874$	$r_{15\cdot3} = +\cdot4758$	$r_{15\cdot34} = +\cdot2567$	$r_{15\cdot234} = +\cdot1988$
		$r_{15\cdot32} = +\cdot2312$	
	$r_{15\cdot4} = +\cdot3925$	$r_{15\cdot42} = +\cdot1849$	
		$r_{15\cdot43} = +\cdot2567$	

TABLE—*contd*

B_4

$r_{12} = -·3662$	$r_{12·3} = -·1115$	$r_{12·34} = -·0$[illegible]5[illegible]	$r_{12·345} = -·0$[illegible]72
		$r_{12·35} = -·0174$	
	$r_{12·4} = -·2459$	$r_{12·45} = -·1983$	
		$r_{12·43} = -·0$[illegible]52	
	r_{1}[illegible]$= -·1919$	$r_{12·53} = -·0174$	
		$r_{12·54} = -·1983$	
$r_{13} = -·4493$	$r_{13·2} = -·2996$	$r_{13·24} = -·3098$	$r_{13·245} = -·3098$
		$r_{13·25} = -·3028$	
	$r_{13·4} = -·3874$	$r_{13·45} = -·3618$	
		$r_{13·42} = -·3098$	
	$r_{13·5} = -·3533$	$r_{13·52} = -·30$[illegible]8	
		$r_{13·54} = -·3618$	
$r_{14} = -·2986$	$r_{14·2} = -·1083$	$r_{14·23} = -·1357$	$r_{14·235} = -·0877$
		$r_{14·25} = -·0552$	
	$r_{14·3} = -·1733$	$r_{14·35} = -·0851$	
		$r_{14·32} = -·1357$	
	$r_{14·5} = -·0181$	$r_{14·52} = -·0552$	
		$r_{14·53} = -·0851$	
$r_{15} = +·3301$	$r_{15·2} = +·0939$	$r_{15·23} = +·1044$	$r_{15·234} = -·0108$
		$r_{15·24} = +·0100$	
	$r_{15·3} = +·1515$	$r_{15·34} = +·0024$	
		$r_{15·32} = +·1044$	
	$r_{15·4} = +·1487$	$r_{15·42} = +·0100$	
		$r_{15·43} = +·00$[illegible]4	

TABLE—*contd*

B. & O.

$r_{12} = +\cdot4417$	$r_{12\cdot3} = +\cdot4059$ $r_{12\cdot4} = +\cdot2307$ $r_{12\cdot5} = +\cdot0623$	$r_{12\cdot34} = +\cdot0541$ $r_{12\cdot35} = -\cdot0360$ $r_{12\cdot45} = +\cdot0824$ $r_{12\cdot43} = +\cdot0541$ $r_{12\cdot53} = -\cdot0360$ $r_{12\cdot54} = +\cdot0824$	$r_{12\cdot345} = -\cdot0611$
$r_{13} = +\cdot2232$	$r_{13\cdot2} = -\cdot1181$ $r_{13\cdot4} = +\cdot2484$ $r_{13\cdot5} = +\cdot1103$	$r_{13\cdot24} = +\cdot1088$ $r_{13\cdot25} = +\cdot0979$ $r_{13\cdot45} = +\cdot1572$ $r_{13\cdot42} = +\cdot1068$ $r_{13\cdot52} = +\cdot0979$ $r_{13\cdot54} = +\cdot1572$	$r_{13\cdot245} = +\cdot1473$
$r_{14} = +\cdot5009$	$r_{14\cdot2} = +\cdot3448$ $r_{14\cdot3} = +\cdot5101$ $r_{14\cdot5} = +\cdot1112$	$r_{14\cdot23} = +\cdot3421$ $r_{14\cdot25} - +\cdot1234$ $r_{14\cdot35} = +\cdot1576$ $r_{14\cdot32} = +\cdot3421$ $r_{14\cdot52} = +\cdot1234$ $r_{14\cdot53} = +\cdot1576$	$r_{14\cdot235} = +\cdot1653$
$r_{15} = -\cdot5457$	$r_{15\cdot2} = -\cdot3619$ $r_{15\cdot3} = -\cdot5196$ $r_{15\cdot4} = -\cdot2723$	$r_{15\cdot23} = -\cdot3566$ $r_{15\cdot24} = -\cdot1696$ $r_{15\cdot34} = -\cdot1941$ $r_{15\cdot32} = -\cdot3566$ $r_{15\cdot42} = \quad \cdot169$[illegible] $r_{15\cdot43} - -\cdot1941$	$r_{1^5\cdot234} = -\cdot1962$

TABLE—*contd*

U. P.

	$r_{12.3} = +.2272$	$r_{12.34} = -.1751$	
		$r_{12.35} = -.0588$	
$r_{12} = +.2321$	$r_{12.4} = +.0773$	$r_{12.45} = +.0646$	$r_{12.345} = -.1688$
		$r_{12.43} = -.1751$	
	$r_{12.5} = +.0319$	$r_{12.53} = -.0588$	
		$r_{12.54} = +.0646$	
	$r_{13.2} = -.0697$	$r_{13.24} = +.2650$	
		$r_{13.25} = +.1062$	
$r_{13} = +.0851$	$r_{13.4} = +.2158$	$r_{13.45} = +.2167$	$r_{13.245} = +.2649$
		$r_{13.42} = +.2650$	
	$r_{13.5} = +.0941$	$r_{13.52} = +.1062$	
		$r_{13.54} = +.2167$	
	$r_{14.2} = +.3389$	$r_{14.23} = +.4159$	
		$r_{14.25} = +.2083$	
$r_{14} = +.3969$	$r_{14.3} = +.4369$	$r_{14.35} = +.2780$	$r_{14.235} = +.3168$
		$r_{14.32} = +.4159$	
	$r_{14.5} = +.2010$	$r_{14.52} = +.2083$	
		$r_{14.53} = +.2780$	
	$r_{15.2} = -.2734$	$r_{15.23} = -.2841$	
		$r_{15.24} = -.0074$	
$r_{15} = -.3517$	$r_{15.3} = -.3537$	$r_{15.34} = +.0474$	$r_{15.234} = -.0037$
		$r_{15.32} = -.2841$	
	$r_{15.4} = -.0432$	$r_{15.42} = -.0074$	
		$r_{15.43} = +.0474$	

TABLE—*contd*

C. P.

	$r_{12\cdot3} = + 3780$	$r_{12\cdot34} = + 1638$	
		$r_{12\cdot35} = + 1062$	
$r_{12} = + 3567$	$r_{12\cdot4} = + 2987$	$r_{12\cdot45} = + 1544$	$r_{12\cdot345} = + 1063$
		$r_{12\cdot43} = +\cdot1638$	
	$r_{12\cdot5} = + 1254$	$r_{12\cdot53} = + 1062$	
		$r_{12\cdot54} = + 1$[illegible]44	
	$r_{13\cdot2} = - 1779$	$r_{13\cdot24} = +\cdot0076$	
		$r_{13\cdot25} = - 0366$	
$r_{13} = + 1951$	$r_{13\cdot4} = + 253$[illegible]	$r_{13\cdot45} = + 1115$	$r_{13\cdot245} = + 0013$
		$r_{13\cdot42} = +\cdot0076$	
	$r_{13\cdot5} = + 0$[illegible]63	$r_{13\cdot52} = - 0366$	
		$r_{13\cdot54} = + 1115$	
	$r_{14\cdot2} = + 2295$	$r_{14\cdot23} = + 19$[illegible]1	
		$r_{14\cdot25} = + 0934$	
$r_{14} = + 3038$	$r_{14\cdot3} = + 3418$	$r_{14\cdot35} = + 0845$	$r_{14\cdot235} = + 08$[illegible]9
		$r_{14\cdot32} = + 19$[illegible]1	
	$r_{14\cdot5} = + 0227$	$r_{14\cdot52} = + 0934$	
		$r_{14\cdot53} = + 0845$	
	$r_{15\cdot2} = - 2211$	$r_{15\cdot23} = - 1953$	
		$r_{15\cdot24} = -\cdot0689$	
$r_{15} = - 3958$	$r_{15\cdot3} = - 3$[illegible]63	$r_{15\cdot34} = -\cdot1437$	$r_{15\cdot234} = -\cdot0688$
		$r_{15\cdot32} = -\cdot1953$	
	$r_{15\cdot4} = - 2672$	$r_{15\cdot42} = -\cdot0689$	
		$r_{15\cdot43} = - 1437$	

TABLE—*contd*

U. P.

$r_{12} = +\cdot 2321$	$r_{12.3} = +\cdot 2272$	$r_{12.34} = -\cdot 1751$ $r_{12.35} = -\cdot 0588$	$r_{12.345} = -\cdot 1688$
	$r_{12.4} = +\cdot 0773$	$r_{12.45} = +\cdot 0646$ $r_{12.43} = -\cdot 1751$	
	$r_{12.5} = +\cdot 0319$	$r_{12.53} = -\cdot 0588$ $r_{12.54} = +\cdot 0646$	
$r_{13} = +\cdot 0851$	$r_{13.2} = -\cdot 0697$	$r_{13.24} = +\cdot 2650$ $r_{13.25} = +\cdot 1062$	$r_{13.245} = +\cdot 2649$
	$r_{13.4} = +\cdot 2158$	$r_{13.45} = +\cdot 2167$ $r_{13.42} = +\cdot 2650$	
	$r_{13.5} = +\cdot 0941$	$r_{13.52} = +\cdot 1062$ $r_{13.54} = +\cdot 2167$	
$r_{14} = +\cdot 3969$	$r_{14.2} = +\cdot 3389$	$r_{14.23} = +\cdot 4159$ $r_{14.25} = +\cdot 2083$	$r_{14.235} = +\cdot 3168$
	$r_{14.3} = +\cdot 4369$	$r_{14.35} = +\cdot 2780$ $r_{14.32} = +\cdot 4159$	
	$r_{14.5} = +\cdot 2010$	$r_{14.52} = +\cdot 2083$ $r_{14.53} = +\cdot 2780$	
$r_{15} = -\cdot 3517$	$r_{15.2} = -\cdot 2734$	$r_{15.23} = -\cdot 2841$ $r_{15.24} = -\cdot 0074$	$r_{15.234} = -\cdot 0037$
	$r_{15.3} = -\cdot 3537$	$r_{15.34} = +\cdot 0474$ $r_{15.32} = -\cdot 2841$	
	$r_{15.4} = -\cdot 0432$	$r_{15.42} = -\cdot 0074$ $r_{15.43} = +\cdot 0474$	

TABLE—*contd*

C. P

	r12·3 = + ·3780	r12·34 = + ·1638 r12·35 = + ·1062	
r12 = + ·3567	r12·4 = + ·2987	r12·45 = + ·1544 r12·43 = + ·1638	r12·345 = + ·1063
	r12·5 = + ·1254	r12·53 = + ·1062 r12·54 = + ·1544	
	r13·2 = − ·1779	r13·24 = + ·00[illegible]6 r13·25 = − ·0366	
r13 = + ·1951	r13·4 = + ·253[illegible]	r13·45 = + ·1115 r13·42 = + ·0076	r13·245 = + ·0013
	r13·5 = + ·0763	r13·52 = − ·0366 r13·54 = + ·1115	
	r14·2 = + ·2795	r14·23 = + ·1971 r14·25 = + ·0934	
r14 = + ·3038	r14·3 = + ·3418	r14·35 = + ·0845 r14·32 = + ·1971	r14·235 = + ·0859
	r14·5 = + ·0[illegible]7	r14·52 = + ·0934 r14·53 = + ·0845	
	r15·2 = − ·2[illegible]11	r15·23 = − ·1853 r15·24 = − ·0689	
r15 = − ·3958	r15·3 = − ·3583	r15·34 = − ·1437 r15·32 = − ·1853	r15·234 = − ·0688
	r15·4 = − ·2672	r15·42 = − ·0689 r15·43 = − ·1437	

TABLE—*contd*

P_r

	$r_{12\cdot3} = +\cdot4702$	$r_{12\cdot34} = +\cdot1428$	
		$r_{12\cdot35} = +\cdot1337$	
$r_{12} = +\cdot4228$	$r_{12\cdot4} = +\cdot3020$	$r_{12\cdot45} = +\cdot2450$	$r_{12\cdot345} = +\cdot1207$
		$r_{12\cdot43} = +\cdot1428$	
	$r_{12\cdot5} = +\cdot2395$	$r_{12\cdot53} = +\cdot1337$	
		$r_{12\cdot54} = +\cdot2450$	
	$r_{13\cdot2} = -\cdot2270$	$r_{13\cdot24} = +\cdot0526$	
		$r_{13\cdot25} = +\cdot0707$	
$r_{13} = +\cdot0091$	$r_{13\cdot4} = +\cdot2736$	$r_{13\cdot45} = +\cdot2206$	$r_{13\cdot245} = +\cdot0518$
		$r_{13\cdot42} = +\cdot0526$	
	$r_{13\cdot5} = +\cdot2016$	$r_{13\cdot52} = +\cdot0707$	
		$r_{13\cdot54} = +\cdot2206$	
	$r_{14\cdot2} = +\cdot3139$	$r_{14\cdot23} = +\cdot2285$	
		$r_{14\cdot25} = +\cdot0577$	
$r_{14} = +\cdot4306$	$r_{14\cdot3} = +\cdot4964$	$r_{14\cdot35} = +\cdot0946$	$r_{14\cdot235} = +\cdot0746$
		$r_{14\cdot32} = +\cdot2285$	
	$r_{14\cdot5} = -\cdot0233$	$r_{14\cdot52} = +\cdot0577$	
		$r_{14\cdot53} = +\cdot0946$	
	$r_{15\cdot2} = -\cdot3141$	$r_{15\cdot23} = -\cdot2239$	
		$r_{15\cdot24} = -\cdot0589$	
$r_{15} = -\cdot4634$	$r_{15\cdot3} = -\cdot4966$	$r_{15\cdot34} = -\cdot0963$	$r_{15\cdot234} = -\cdot0581$
		$r_{15\cdot32} = -\cdot2239$	
	$r_{15\cdot4} = -\cdot1910$	$r_{15\cdot42} = -\cdot0589$	
		$r_{15\cdot43} = -\cdot0963$	

TABLE—*contd*

B,

	$r_{12\cdot3} = +\cdot1922$	$r_{12\cdot34} = +\cdot1761$	
		$r_{12\cdot35} = +\cdot0991$	
$r_{12} = +\cdot4215$	$r_{12\cdot4} = +\cdot3163$	$r_{12\cdot45} = +\cdot1585$	$r_{12\cdot345} = +\cdot1144$
		$r_{12\cdot43} = +\cdot1761$	
	$r_{12\cdot5} = +\cdot1439$	$r_{12\cdot53} = +\cdot0991$	
		$r_{12\cdot54} = +\cdot1585$	
	$r_{13\cdot2} = +\cdot1088$	$r_{13\cdot24} = +\cdot0610$	
		$r_{13\cdot25} = +\cdot0408$	
$r_{13} = +\cdot3950$	$r_{13\cdot4} = +\cdot2731$	$r_{13\cdot45} = +\cdot1170$	$r_{13\cdot245} = +\cdot0401$
		$r_{13\cdot42} = +\cdot0610$	
	$r_{13\cdot5} = +\cdot1121$	$r_{13\cdot52} = +\cdot0408$	
		$r_{13\cdot54} = +\cdot1170$	
	$r_{14\cdot2} = +\cdot2065$	$r_{14\cdot23} = +\cdot1864$	
		$r_{14\cdot25} = +\cdot0696$	
$r_{14} = +\cdot3539$	$r_{14\cdot3} = +\cdot2019$	$r_{14\cdot35} = +\cdot0386$	$r_{14\cdot235} = +\cdot0697$
		$r_{14\cdot32} = +\cdot1864$	
	$r_{14\cdot5} = +\cdot0191$	$r_{14\cdot52} = +\cdot0696$	
		$r_{14\cdot53} = +\cdot0386$	
	$r_{15\cdot2} = -\cdot2184$	$r_{15\cdot23} = -\cdot1946$	
		$r_{15\cdot24} = -\cdot1005$	
$r_{15} = -\cdot4476$	$r_{15\cdot3} = -\cdot2537$	$r_{15\cdot34} = -\cdot1614$	$r_{15\cdot234} = -\cdot0894$
		$r_{15\cdot32} = -\cdot1946$	
	$r_{15\cdot4} = -\cdot2936$	$r_{15\cdot42} = -\cdot1005$	
		$r_{15\cdot43} = -\cdot1614$	

TABLE—*contd*

M_1

	$r_{12\cdot3} = +2615$	$r_{12\cdot34} = +0800$ $r_{12\cdot35} = -0367$	
$r_{12} = +3788$	$r_{12\cdot4} = +3453$	$r_{12\cdot45} = +1353$ $r_{12\cdot43} = +0800$	$r_{12\cdot345} = -\cdot0139$
	$r_{12\cdot5} = +1877$	$r_{12\cdot53} = -0367$ $r_{12\cdot54} = +1353$	
	$r_{13\cdot2} = +0212$	$r_{13\cdot24} = +1763$ $r_{13\cdot25} = +1887$	
$r_{13} = +2847$	$r_{13\cdot4} = +3573$	$r_{13\cdot45} = +1788$ $r_{13\cdot42} = +1763$	$r_{13\cdot245} = +1188$
	$r_{13\cdot5} = +2614$	$r_{13\cdot52} = +1887$ $r_{13\cdot54} = +1788$	
	$r_{14\cdot2} = +0948$	$r_{14\cdot23} = +1562$ $r_{14\cdot25} = -1624$	
$r_{14} = +1906$	$r_{14\cdot3} = +2970$	$r_{14\cdot35} = -\cdot0761$ $r_{14\cdot32} = +1562$	$r_{14\cdot235} = -0681$
	$r_{14\cdot5} = -2078$	$r_{14\cdot52} = -1674$ $r_{14\cdot53} = -\cdot0761$	
	$r_{15\cdot2} = -2587$	$r_{15\cdot23} = -3158$ $r_{15\cdot24} = -2885$	
$r_{15} = -4143$	$r_{15\cdot3} = -4003$	$r_{15\cdot34} = -\cdot2954$ $r_{15\cdot32} = -3158$	$r_{15\cdot234} = -\cdot2854$
	$r_{15\cdot4} = -4214$	$r_{15\cdot42} = -2885$ $r_{15\cdot43} = -\cdot2954$	

TABLE—*contd*

M_2

	$r_{12\cdot3} = -\cdot2674$	$r_{12\cdot34} = -\cdot1266$	
		$r_{12\cdot35} = -\cdot1135$	
$r_{12} = +\cdot0265$	$r_{12\cdot4} = -\cdot1082$	$r_{12\cdot45} = -\cdot1177$	$r_{12\cdot345} = -\cdot1811$
		$r_{12\cdot43} = -\cdot1266$	
	$r_{12\cdot5} = +\cdot0430$	$r_{12\cdot53} = -\cdot1135$	
		$r_{12\cdot54} = -\cdot1177$	
	$r_{13\cdot2} = +\cdot4707$	$r_{13\cdot24} = +\cdot0674$	
		$r_{13\cdot25} = +\cdot1641$	
$r_{13} = +\cdot4027$	$r_{13\cdot4} = -\cdot0152$	$r_{13\cdot45} = -\cdot0132$	$r_{13\cdot245} = +\cdot1388$
		$r_{13\cdot42} = +\cdot0674$	
	$r_{13\cdot5} = +\cdot1265$	$r_{13\cdot52} = +\cdot1641$	
		$r_{13\cdot54} = -\cdot0132$	
	$r_{14\cdot2} = -\cdot5727$	$r_{14\cdot23} = -\cdot3750$	
		$r_{14\cdot25} = -\cdot3919$	
$r_{14} = -\cdot5660$	$r_{14\cdot3} = -\cdot4348$	$r_{14\cdot35} = -\cdot3599$	$r_{14\cdot235} = -\cdot3835$
		$r_{14\cdot32} = -\cdot3750$	
	$r_{14\cdot5} = -\cdot3784$	$r_{14\cdot52} = -\cdot3919$	
		$r_{14\cdot53} = -\cdot3599$	
	$r_{15\cdot2} = +\cdot4558$	$r_{15\cdot23} = +\cdot0981$	
		$r_{15\cdot24} = -\cdot0474$	
$r_{15} = +\cdot4548$	$r_{15\cdot3} = +\cdot2617$	$r_{15\cdot34} = -\cdot0043$	$r_{15\cdot234} = -\cdot1306$
		$r_{15\cdot32} = +\cdot0981$	
	$r_{15\cdot4} = -\cdot0087$	$r_{15\cdot42} = -\cdot0474$	
		$r_{15\cdot43} = -\cdot0043$	

When in 1902 information reached the island that cholera was raging in Canton Hongkong and Manila the Governor General issued prevention rules and established temporary quarantine stations at Keelung Takao Tamsui and Amping and endeavoured to check the epidemic by a strict quarantine Nevertheless a case of unknown origin broke out in Taihoku city on 15th May and the cholera spread everywhere invading the majority of the 22 prefectures at that time In all 746 cases were reported 613 of which ended in death However this epidemic was eradicated in December of that year after much trouble

In 1912 cholera ravaged every place on the China coast especially such places as Shanghai and Foochow On 10th June cholera of unknown origin appeared aboard a fishing boat then in the port of Keelung and in spite of the Authorities having kept and keeping the strictest watch as we are in frequent and constant communication with those districts the pestilence spread to other vessels in the harbour as well as to the town itself and many cases appeared On 13th June it attacked Taihoku and Giran very severely and its aftermath reached to Shirin Toen Shinchiku Taichu Karenko and other places In December however it subsided after having caused 333 cases

In 1919 the Authorities received news that cholera had made its appearance on the South China coast especially in the districts of Swatow and Foochow In these circumstances a crisis was almost imminent as we are separated from the mainland only by a narrow strait In addition to this as we had had the bitter experience of an attack of cholera every year the Authorities were keeping a stricter watch On 7th July however a case of cholera of unascertainable origin appeared *in Boko prefecture and on the 8th of that month the s s Kokoku Maru brought* a case from Foochow to Keelung moreover several cases appeared in Hozan sub prefecture thus cholera spread very rapidly all over the island The Authorities did their best to prevent further spreading but in spite of all their efforts 3 836 cases occurred of which 2 689 deaths were reported It is the strangest fact when 500 or more cases of cholera broke out in the village of Marabansha a savage village in Taito prefecture that all the villagers horror stricken ran away from their houses to other places in the forests of the mountains This terrible tragedy ended in December 1919 and people were greatly relieved for the time

At the beginning of the hot season of 1920 the incubated virus of the previous *year raised its head In the early part of April more than ten cases happened* in Takao province in the course of a few days cholera reached Okayama district and Tainan city and spread all over that province with irresistible force The virus actually overran the province Ultimately it came to Taichu province and its effects extended to Taichu city But by the Authorities proper acts for its prevention it was fortunately stamped out However its destructive power in Taichu city and in Tainan province was overwhelming The general disaster equally balanced that of the previous year Thus the total number of deaths was calculated at 1 675 out of 2 670 cases In January of the following year however it had entirely disappeared

From the above we can now easily understand that epidemics of cholera in Japan proper, in South China and South Seas are really the root of its prevalence in this island

II Special Points re the Prevention of Epidemics in Formosa

(a) *Idea of Sanitation in Formosa* —At the time of the Japanese occupation of Formosa, the people had no proper idea of sanitation and the island was quite an unhealthy place The Authorities have adopted the policy of a gradual prohibition of opium and provided sanitary arrangements such as water supply sewage street improvements and markets so that they might establish a foundation for sanitation The port quarantine system has been laid down in order to exclude infectious diseases and building regulations came into effect to stamp out the bubonic plague They also devised a means for the extermination of malaria In connection with sanitary agencies they established Government hospitals in the cities and for the training of doctors they opened a medical college As a result the sanitary conditions in the island have greatly improved By the weight and influence of the above arrangements directly or indirectly the Formosans individual sanitary idea has considerably advanced to say nothing of the impetus lent to the sanitary idea of the public in general yet in most of this only the outward letter is attained by the system and owing to the spontaneity of the public there still is vast space for improvement The Formosan race is according to conception of its nature indifferent to sanitation It is rather a characteristic of theirs Such low sanitary ideas have been perfectly manifest in every place where cholera prevailed Two or three practical examples may illustrate this—

Examples —When cholera raged in Taihoku city and other places, its aftermath extended to Shinten sub prefecture where one case was reported The prefecture presented an extraordinary state of fear among the people because the virus had spread all over the banks of the down stream but they had thrown the patient s excretions into their irrigation Furthermore there was a tragedy in Sankakuyu sub prefecture Toen prefecture At that time as soon as signs of the prevalence of cholera appeared in that locality the prefectural Authorities endeavoured to locate patients carrying out a house to house examination of the inhabitants who were suspected of spreading the disease It is a fact that in a certain house a patient was hidden in the corner of the kitchen under a pile of accumulated fire wood so that no sound from him might be heard but he was discovered On one occasion they were taking patients out through the back doors of their houses on the sly when the officials came to visit them and concealed their sick in a ravine situated several thousand yards away

Some patients escaped in the dark from their houses by a river boat or palanquin and went to their native places This was the greatest danger at the time of the prevalence of this disease

There are many such practical examples They do not know what 'carrier' means, they cannot understand why patients and carriers must be isolated, they

hate the idea of having to go into isolation hospitals or to be put in quarantine When a case suddenly occurs the healthy people try to get away from the patient's house secretly or will wash up the patient's vomitings and excretions and even hide the patients themselves Therefore the Government experienced much trouble and inconvenience in locating patients

(*b*) *Customs* —The Formosans individual sanitary idea is the same as that of the Chinese race generally except for the two or three intellectual classes They are accustomed to treat illness by charm or prayer irrespective of its character as an infectious or a general disease There remains still a small number which receives medical treatment and uses Chinese medicines If they die nobody wonders in the least they resign themselves to fate Most of the Formosans who are addicted to such practices sometimes when patients are reported conceal them or provide a great many opportunities for infection by having relatives and friends gathered in the patient's house to comfort the sick man one after another they throng around the dangerously sick and dying patient and themselves sit on the same seats dine at the same tables eat the same food and so forth Such evil customs and this wrong moral sense of several hundred years standing are very difficult to prevent in days of prevalence

Practical Examples —When cholera raged in Taihoku city and other places it finally attacked Shinsho where one case appeared Most of the inhabitants of the town thought that they could prevent the epidemic by supplicating divine protection but that it could not be helped They secretly brought the image of Rakubi Soshi from the Manka Soshi Shrine and enshrined it in the shrine of the patient's house where they prayed for the dispersion of the disease making an offering to the God Then prayers over they gave the offering to the patient whereby they believed that the patient would be restored to health Moreover all who had attended the service dined coolly with the patient There are many instances like that stated above and that such superstitious customs have been great obstacles in the prevention work of the Authorities goes without saying

(*c*) *Quarantine and Preventive Measures against Cholera in Formosa* —General information regarding the epidemic situation in South China the South Sea islands and in the neighbouring countries is obtained through the Consular reports or through the directors of the Hakuai hospitals stationed at certain important seaports such as Foochow Amoy Swatow and Canton

Of maritime quarantine stations in Formosa there are at present two permanent ones viz at Takao and Keelung and a branch office of the Keelung quarantine station at Tamsui

The Keelung quarantine station in Formosa is the only one equipped with a detention house disinfecting plants isolation hospital and other necessary buildings for execution of efficient quarantine measures When a cholera epidemic in one of the seaports of the neighbouring countries becomes severe that port is proclaimed as a cholera infected port A search for bacilli carriers is then started

and the fæces of passengers and crews of all the vessels arriving from the infected port examined To carry out these preventive measures, takes about eight or ten hours and for that time the vessels are detained

In Formosa as well as in Japan cholera, like other acute infectious diseases is controlled by law and bacilli carriers are looked upon as true cases of cholera

As soon as a case of cholera is reported the Quarantine or Health Officer the Police Officer the City and Town officials make an inspection and the patient is promptly removed to the isolation hospital the premises are disinfected and those exposed to contagion are interned either in their houses or some other appropriate place *Meantime the route of the infection is minutely investigated* When river or seawater is found to be the media of infection the use of that water for fishing or swimming is prohibited And then the entire population of a district in which many cholera cases have been reported receives prophylactic inoculation of vaccine and fæcal examinations are made two or three times

Conclusions

(1) Formosa lying in such close geographical situation of South China carrying on an incessant trade by steamers and junks with that country and resulting even in congestion of traffic in the island the conclusion that the virus in this way and in most cases, came to this island is not far fetched though there are a few exceptions

(2) The cholera epidemic in this island periodically occurs every seven or ten years and it goes in parallel with the prevalence in South China the South Sea islands and other countries and we can easily understand that the epidemics in the China coast are really the root of its prevalence in this island

(3) In regard to the prevalence of this disease in the island it is mainly due to the evil habit and customs to which native families still adhere and which are bound to spread the infection such as to live in close contact with the sick etc As to the route of cholera infection may also be found in a few cases of food infection notwithstanding Formosans always drank boiled water and ate boiled food

TABLE I

Showing the Cholera Epidemics in Formosa since 1895

Year	First case	Route of invasion	End	Number of cases	Number of deaths
1895	End of March	From Japan	End of May	21 945	1 247
1898				Japanese 1	
1901				Formosan 1	1

TABLE I—*concld*

Year	First case	Route of invasion	End.	Number of cases		Number of deaths
1902	On 15th May	There was a severe epidemic at Canton Hongkong and Manila in this year	Beginning of December	Japanese	202	131
				Formosan	544	479
1904				Formosan	1	1
1907	On 27th August	From Japan	Middle of December	Japanese	3	2
1910				Japanese	13	8
1912	On 10th June	There was a severe epidemic in Shanghai and Foochow districts	End of December	Japanese	121	70
				Formosan	212	166
1916	On 28th September	From the South Seas there was a severe epidemic in Java and China that year	Middle of May (1927)	Japanese	32	16
				Formosan	2	1
1917				Japanese	2	1
1918				Japanese	1	1
1919	On 7th July	From Foochow	On 26th November	Japanese	149	87
				Formosan	4 358	3 176
1920	On 10th April	Virus remained from last year		Formosan	1 270	880
1925	On 2nd October	From Amoy	On 31st October	Japanese	3	3
1926	On 31st August	From Foochow	On 18th October	Japanese	2	1
				Formosan	13	8
				Chinese	1	

TABLE II

Showing the Progressive Course of Cases

Day	Race	Cases		Cured		Deaths	
		1919	1920	1919	1920	1919	1920
Within 1 day	Japanese	32	16			32	16
	Formosan	1,576	1,379	1		1 575	1 379
	Chinese	39	3			39	3
Within 5 days	Japanese	63	5	12	1	51	4
	Formosan	852	177	66	26	786	151
	Chinese	20	1			20	1
Within 10 days	Japanese	34	15	21	12	13	3
	Formosan	460	311	325	266	135	45
	Chinese	2		1		1	
Within 15 days	Japanese	24	16	23	15	1	1
	Formosan	377	309	356	282	21	27
	Chinese	4		3		1	
Within 20 days	Japanese	17	11	16	8	1	3
	Formosan	235	206	222	197	13	9
	Chinese	2		2			
Over 20 days	Japanese	11		11			
	Formosan	86	221	83	188	3	33
	Chinese	1		1			
TOTAL	Japanese	181	63	83	36	98	27
	Formosan	3 586	2,603	1 053	959	2 533	1 644
	Chinese	68	4	7		61	4

Table III

Showing the Sex of Cases

Race	Sex	Cases		Cured		Deaths	
		1919	1920	1919	1920	1919	1920
Japanese	Male	119	52	61	33	58	19
	Female	62	11	22	3	48	8
Formosan	Male	1 833	1 178	507	4 3	1 326	755
	Female	1 753	1 425	546	536	1 207	889
Chinese	Male	63	4	6		57	4
	Female	6		1		5	
Total	Male	2 015	1 235	574	456	1 441	778
	Female	1 821	1 435	569	539	1 252	897

Table IV

Showing the Age of Cases

Age	Race	Cases		Cured		Deaths	
		1919	1920	1919	1920	1919	1920
Under 5 years	Japanese	10		7		3	
	Formosan	347	276	100	74	247	202
	Chinese	2		1		1	
10 years	Japanese	10		5		5	
	Formosan	376	338	130	136	246	203
	Chinese						

TABLE IV—*concld*

Age.	Race	Cases 1919	Cases 1920	Cured 1919	Cured 1920	Deaths 1919	Deaths 1920
15 years	Japanese	9	1	2		7	1
	Formosan	209	173	101	83	108	90
	Chinese						
20 years	Japanese	6	1	5		1	1
	Formosan	228	189	136	97	92	92
	Chinese						
30 years	Japanese	45	31	13	24	32	7
	Formosan	625	466	237	225	388	241
	Chinese	16		1		15	--
40 years	Japanese	47	18	23	2	24	11
	Formosan	602	476	105	195	437	281
	Chinese	23	3	3		20	3
50 years	Japanese	31	8	13	4	18	4
	Formosan	464	367	100	116	364	251
	Chinese	19	1	2		17	
Under 60 years	Japanese	15	6	6	3	9	3
	Formosan	399	198	57	52	342	146
	Chinese	5	5			5	
Under 70 years	Japanese	5	5			5	
	Formosan	244	119	20	18	224	101
	Chinese	3				3	
Over 70 years	Japanese	3				3	
	Formosan	92		7	8	85	47
	Chinese	1				1	

Table V

Showing the Occupation of Cases

Occupation	Race	Cases		Cured		Deaths	
		1919	1920	1919	1920	1919	1920
Agriculture forestry and farming	Japanese	3		1		2	
	Formosan	1 679	1 560	55[illegible]	554	1 127	1 006
	Chinese						
Fishing salt manufacturing	Japanese	12	1	1		11	1
	Formosan	578	18[illegible]	[illegible]53	83	3[illegible]5	99
	Chinese						
Industry	Japanese	19	1	6		13	1
	Formosan	64	37	8	13	56	24
	Chinese	8		1		7	
Commerce and traffic manufacturing	Japanese	43	3	[illegible]3		20	3
	Formosan	248	95	46	33	202	6[illegible]
	Chinese	[illegible]2		1		21	
Public service and other business	Japanese	10[illegible]	57	52	36	50	21
	Formosan	931	728	158	[illegible]76	773	45[illegible]
	Chinese	38	4	5		33	4
No occupation	Japanese	2	1			[illegible]	1
	Formosan	86	1	36		50	1
	Chinese						
Total	Japanese	181	63	83	36	98	27
	Formosan	3 586	2 603	1 053	959	[illegible] 533	1 644
	Chinese	68	4	7		61	4

TABLE VI

Showing Cholera Carriers (1920)

Race	Number of carriers	Number of carriers becoming cases	Number of persons having received inoculation	Number of persons without inoculation
Japanese	39	2	20	19
Formosan	3 006	51	2,054	952
Savage	10			10
TOTAL	3 055	53	2 074	981

TABLE VII

Showing the Period of Discharging Vibrio from the Bacilli Carriers (1920)

Race	Sex	DAY							
		1st—5th	6th—10th	11th—15th	16th—20th	21st—25th	26th—30th	31st—35th	36th—40th
Japanese	Male	5	11	2		1	2		
	Female	6	5	2	2		1		
Formosan	Male	237	480	300	194	124	37		6
	Female	287	547	355	170	77	50		9
Savage	Male		2	3		3		16	
	Female		1	1				11	
TOTAL	Male	242	493	305	194	128	39	16	6
	Female	293	553	358	172	77	51	11	9

Table VIII

Showing the Number of Cholera Vaccine Inoculations (1920)

Province	Population			Number of Inoculations			Percentage of Inoculations		
	Male	Female	Total	Male	Female	Total	Male	Female	Averag
Taihoku	40 246	358 04[illegible]	760 502	132 127	85 102	217 2[illegible]9	3[illegible] 9	[illegible]3 7	28 5
Shinchiku	295 868	28[illegible] 836	578 704	18 860	14 899	33 759	63 8	5[illegible] 6	58 3
Taichu	406 526	378 448	784 974	153 574	127 485	281 059	37 8	33 7	35 8
Tainan	41[illegible] 101	384 333	796 434	260 742	2[illegible]6 464	487 506	65 6	59 0	61 2
Takao	118 [illegible]85	107 724	2[illegible]6 009	89 964	75 863	165 827	76 0	70·4	73 3
Ta to	3 636	3 255	6 891	3 424	2 515	5 939	[illegible][illegible] 1	74 1	68 9
Karenko	26 692	2[illegible] 605	49 [illegible]97	14 154	10 377	2 454	53 0	45 9	49 7
Total	1 665 568	1 537 [illegible]42	3 [illegible]0 810	672 845	543 016	1 [illegible]15 861	40 4	35 3	37 9

Table IX

Showing the Occurrence of Cases after Inoculation (1920)

Day	Race	Cases	Cured	Deaths
1st	Japanese			
	Formosan	38	10	28
2nd	Japanese			
	Formosan	61	26	35
3rd	Japanese			
	Formosan	72	31	41
4th	Japanese	1		1
	Formosan	59	[illegible]1	38
5th	Japanese	1	1	
	Formosan	84	35	49
6th—10th	Japanese	2	2	
	Formosan	1[illegible]0	47	73

TABLE IX—*concld*

Day	Race	Cases	Cured.	Deaths
11th—15th	Japanese	2	1	1
	Formosan	69	41	28
16th—20th	Japanese	3		3
	Formosan	83	39	44
21st—30th	Japanese	2		2
	Formosan	192	95	97
31st—40th	Japanese	3	2	1
	Formosan	189	75	114
41st—50th	Japanese			
	Formosan	40	16	24
51st—60th	Japanese			
	Formosan	39	18	21
61st—70th	Japanese			
	Formosan	18	7	11
71st—80th	Japanese			
	Formosan	8	4	4
81st—90th	Japanese			
	Formosan	2	1	1
91st—100th	Japanese			
	Formosan	4	1	3
TOTAL	Japanese	14	6	8
	Formosan	1 078	467	611

LA CAMPAGNE ANTICHOLÉRIQUE AU TONKIN, EPIDEMIES DE 1926 1927

PAR

E JOURDRAN

Directeur local de la Sante au Tonkin Docteur es sciences de l Universite de Paris

Le choléra a dans le delta du Tonkin un foyer bien connu Il fait à certaines epoques sous l influence de causes diverses des retours offensifs dans les differentes provinces du protectorat français Il s est montré particulierement sevère pendant les années 1926 et 1927 et il n a pu etre jugulé que par la mise en vigueur de tout un ensemble de mesures prescrites par les autorités administratives et médicales responsables de la protection de la santé publique La lutte a été entreprise par l initiative de la résidence supérieure de l inspection des services sanitaires et médicaux et de la Direction locale de la santé au Tonkin à qui incombait la tache d établir la programme de defense et de prophylaxie génerale contre le fléau

Le comité d hygiene les commissions d hygiene provinciales les bureaux d hygiene urbaine ajoutaent leur concours aux efforts du personnel d rigeant Il importe de faire remarquer combien les organizations particulieres en liaison avec la Direction locale de la santé dans ces circonstances peuvent se donner utilement libre essor par la rapidité d exécution qu ils entrainent et qui sont la rançon du success des mesures prophylactiques c est dans ces conjonctives critiques pendant la période de flottement inévitable qui marque toujours l apparition d un fléau que la decentralisation et la précision des responsabilités individuelles doivent etre assurées Le Japon a montré dans la petite épidemie de choléra qui a sévi en 1924 et 1925 a Kobé la valeur de cette méthode Le personnel de la station quarantenaire de *Kobé reussit à lui seul a enrayer le fléau qui menaçait de s étendre a tout* le Japon et la Direction de la santé publ que au ministere de l Interieur a Tokyo n eut pour ainsi dire pas à intervenir Nous verrons qu au Tonkin la Direction locale a cherché à creer et a définir les attributions des divers rouages sanitaires

Les facteurs qui ont influé sur la réapparition du cholera en 1926 et 1927 dans le delta du fleuve rouge restent un peu obscurs mais tous les médecins attachent de l importance aux inondations qui ont ravagé en 1926 une grande partie du delta amenant apres elles la misere et la famine malgré les secours distribués par l administration et les œuvres philantropiques

Privés de combustible les mon les en furent réduits dans beaucoup d endroits à se nourrir de troncs de bananiers ecrasés et manges crus ou assaisonnes de sel Cette nourriture indigeste est celle des animaux de basse cour elle devait ouvrir la porte à l enterite et favoriser ainsi l action et la diffusion du vibrion de Koch

L impossibilite d enterrer les cadavres ajoutait une autre cause d insalubrite à celles que nous avons exposées

Les émigrations des habitants faméliques en quete de travail comme le fait remarquer Letort dans la vallee du fleuve rouge les exodes des colporteurs des petits commerçants montant du delta au pays de la haute region ont repandu l epidémie jusqu à Soula par Ngia Lo et Dai Lich et Truong Bung La

La saison des fruits verts mangés avant la maturité la promiscuit des loca taires dans les maisons la souillure des aliments par les mouches les repas funeraires ont ajoute encore leur influence nocive aux autres facteurs Il est a remarquer que les villages meos et mans situes au sommet des montagnes ont habituellement éte epargnes ou peu atteintes par l epidemie Le lavage des legumes dans les eaux des mares contaminees par les dejections des choleriques l ingestion de ces legumes presque crus ont certainement facilité la contamination Pour la ville de Haiphong le Dr Forest attribue à la rupture des canalizations d eau potable et a l absorption de l eau des mares qu en fut la conséquence la flambée epidemique de 1926 en 1915 et 1916 avait fait également de nombreuses victimes

Six jours après l accident des conduites d eau la courbe de la morbidité faisait une ascension formidable 55 cas sont signales dans la meme journee et la courbe redescend le 25 Décembre 6 jours apres la remise en état des conduites d eau le choléra se develope surtout à cette periode a l exterieur du réseau de distribution 477 cas dont 417 deces le bilan de l epidem e d Haiphong en 1926—le dernier cas etait signalé le 12 Janvier mais le 3 Avril l epidemie reparaissait dans toute la periphérie de la ville avec 1164 cas et 1039 deces

88 563 vaccinations furent pratiquées à Haiphong et l épidemie fut enrayee Le Dr Marchive pense que l influence de la saison chaude est évidente c est ce qu il a constaté a Soutay

Le fléau frappe surtout les pauvres les surmenés les gens mal nourris les uha que travaillant dans les rizieres les mandarins les petits commerçants installés a demeure dans les villages sont a peu pres indemnes

L entassement la promiscuite sont encore signalés par le Dr Marchive comme des facteurs etiolog ques importants du choléra Le paludisme les atteintes antérieures de dysenterie ou de diarrhée choleriforme méritent d etre mentionnées comme causes prédisposantes

Ainsi que l a declaré la commission sanitaire de Haiphong réunie le 10 Mai 1927 sous la pres dence du résident maire reunion a laquelle assistait le Directeur local de la Santé au Tonkin l epidémie s annoncait comme une calamité publique et devait etre traitée comme telle En plus des armes que nous fourn ssait pour combattre le choléra le décret du 20 Septembre 1919 qui constitue la charte

sanitaire du protectorat et notamment l'article 3 prévoyant la déclaration d'urgence de la situation sanitaire faite par le Gouvernement en plus des mesures edictées par l'arreté du 6 Juillet 1924 il fallait envisager l'application stricte des moyens speciaux de défence contre l'épidemie pour arriver a depister les malades a les isoler a desinfecter les foyers les habitations les vêtements des malades et a vacciner

L'organisation d'un service exceptionnel de défence entrainant la mobilization de tout le personnel médical et l'augmentation des effectifs sanitaires

Enfin il fallait une coordination des moyens d'action administratifs et medicaux

A Hanoi un arreté du 15 Juin 1926 du résident supérieur ordonnait l'execution immédiate des mesures presentés par les reglements sanitaires Dans les villes et les forts des secteurs furent crees ayant chacun a leur tete un médecin français assisté d'un médecin auxillaure indigene d'un personnel infirmier et d'agents sanitaires

Le médecin Directeur du bureau d'hygiene constituait un organisme central appelé à recevoir tous les renseignements émanant de l'extérieur a les condenser et a proposer toutes mesures utiles complémentaires de défence aux autorités administratives Il fallait une entente complete entre le service municipal d'hygiene *la police* sanitaire maritime et le service de Santé civil et militaire cette entente fut realisée d'une facon générale

La Direction locale de la Santé pendant cette periode consacra une grande partie de son activité a la campagne de défence contre l'épidémie chargée de coordonnance les efforts fournis de tous cotés par le personnel sanitaire la Direction locale prete son concours à tous ceux qui officiellement ou librement à titre privé furent sur la breche des la première heure ne demandant qu'à agir avec méthode et a appliquer les instructions de l'autorite responsable La Direction locale aussitot que le danger de l'épidémie menaça le Tonkin et par repercussion les differentes pays de l'union et les ports de l'exterieur se preocupa d'alerter les autorités *sanitaires des autres pays menacés par les moyens que les reglements mettaient en* son pouvoir et en particulier l'article 7 section II du décret du 7 Juin 1902 ainsi conçu

Lorsque plusieurs cas de choléra se sont manifestes et forment un foyer la circonscription peut etre considerée comme contaminée Le comité d'hygiene se réunit aussitot a Hanoi sous la presidence du résident superieur et declara le Tonkin contaminé de choléra Cette assemblée prescrivit la vacination obligatoire pour toute personne entrant a Haiphong ou en sortant pour toute personne arrivant [illegible] sampan [illegible] e

[illegible] i

aux voyageurs quitant le Tonkin pour l'Annam et inversement Un ordre d'urg[illegible] e fut etabli pour la delivrance du vaccin qu'il importait de distribuer sur la ligne d'étapes ou les voyageurs devaient fournir des certificats de vaccination pour ne pas

se trouver arretes aux frontières des autres pays Le vaccin fut donc delivré en première urgence à Haiphong a Hanoi à Nam Dinh a Ninh Binh a la gare frontiere de Binh Son à Hougay sur les chantiers des charbonnages sur les chantiers des digues de Lam Gin et dans les grandes agglomerations mineurs et agricoles A Hanoi un médecin affecté à l epidimiologie parcourut toutes les administrations vaccinant les collectivités europeenes et indigenes les sociétés industrielles les services de la police de la sureté du cadastre la tresorerie l Ecole industrielle les services agricoles les bureaux de la résidence etc etc Pendant ce temps le médecin charge des Ecoles immunisait tous les ecoliers l ordre etait donné de faire un barrage autour des foyers epidémiques en même temps la Direction locale créait un controle de vaccination et adoptait à cet effet un cachet spécial qui devait etre appose sur les pieces d identité les cartes d impot etc Les demandes de vaccin affluaient au bureau technique et étaient immediatement transmises a l Institut Pasteur Plus tard le vaccin etait stocke a la Pharmacie centrale de l assistance et expedié par elle à tous les postes aux administrations et aux autres collectivités d apres les instructions données par la Direction locale Disposant d un personnel specialisé le service de la Pharmacie centrale pouvait assurer l emballage et l expedition rapide du vaccin au moyen de camionnettes automobiles par les trains et les chaloupes et dans les regions montagneuses par les chevaux ou des mulets à la diligence des autorités administratives locales

L'Institut Pasteur fut d abord débordé et ne put pas fournir à tous les besoins Mais invité a faire face à la gravité de la situation il s organisa en personnel et en matériel et cet etablissement scientifique grace à l activité des Drs Bernard Bablet et Menard réussit à fabriquer sur place le vaccin et la verrerie necessaire à sa conservation Dès ce moment l Institut Pasteur put repondre a toutes les demandes et assura le succes de la campagne anticholerique

Des conseils d hygiene furent donnés à la population par voie d affiches en français en chinois et en quoc ngu et ces affiches répandues à profusion furent apposées dans les services les marchés les Ecoles les mairies etc etc Il fut recommande aux résidents de France et aux médecins dans les diverses provinces contaminées de ne pas exagérer les mesures de contrainte qui sont toujours nuisibles mais de multiplier les conseils et d agir par persuasion sur la mentalité de l indigene Il fallait eviter l affolement qui aurait pu faire le vide sur les chantiers par la desertion des ouvriers c est ce qui s etait produit au début dans les charbonnages de Campha Mine Tres rapidement les indigénes se sont rendu compte qu ils avaient plus de sécurité à accepter les mesures qu on leur imposait et specialement la vaccination anticholérique qu à fuir les foyers de choléra en se derobant à l immunisation

L'œuvre prophylactique se resumait dans la vaccination et nous n avons pas craint de réaliser en grand l experience qui avait été aux Philippines ou la population fut immunisée contre le choléra dans la proportion de 45 pour cent Nous étions certains en prenant ces mesures d etre dans la bonne voie puisque nous avions l appui des savants de l Institut Pasteur avec lequel nous avons toujours travaillé dans la plus étroite collaboration Nous étions d accord aussi avec les conclusions de la

commission épidémiologique dans la conference tenue à Paris le 22 Mai, 1926, et qui ont etabli nettement que la vaccination anticholérique est d une efficacité certaine et bien etablie , elle est nettement specifique , elle permet lorsqu'on l'applique systematiquement d'arreter une épidém e commencante et d'eteindre un foyer epidemique à *son eclosion* , *elle peut et doit être employée en milieu epidemique sans* souci de la problematique phase négative , l'experience l'a longuement démontré , c'est aujourd hui la méthode de choix pour prevenir et arreter l'extension du choléra , elle n'empeche pas l'elimination des germes par les porteurs tout en immunisant, mais elle empeche l'eclosion de la maladie dans leur entourage si ce dernier a éte lui même soumis à la vaccination L'immunité conferée par la vaccination dure pratiquement six mois En resumé, la sous commission épidémiologique est d avis que la vaccination anticholérique est aujourd hui un des éléments essentielles de la prophylaxie du choléra Ces conclusions comme nous l'avons dit sont la plateforme sur laquelle repose toute la reglementation instituée par la Direction locale pour lutter contre le cholera au Tonkin et pour en prevenir le retour Ce n'est pas sans *peine parfois que nous avons imposé ces mesures* *L'utilite de vacciner les pelerins* qui dans les regions d'Hadong et de Nimbinh peuvent constituer un veritable danger en dispersant les foyers de choléra a éte contestée par des personnalités incompetentes malgré notre avis et nous avons du laisser à l'administration locale toute la responsabilité de son obstruction dans la matiere On a aussi insinué que l'obligation des vaccinations était excessive d'une façon generale , que cette méthode n'etait pas anodine , qu'elle entrainait a sa suite quelques accidents fâcheux des nephrites albuminuriques graves et meme quelques cas de mort— nous n'hésitons pas a faire justice a ces critiques Qu'il y ait eu des insuccès chez les sujets fatigués en état de moindre resistance ou presentant des tares telles que paludisme, opiomanie etc , nous n'en disconvenons pas Que des sujets déja en periode d'incubation possible *n'aient pas été* proteges par la vaccination qu'il se soit produit chez eux un shock avant l'apparition des anticorps dans leur organisme, les faits semblent l'établir Mais ce serait faire le sophisme connu sous la formule ' post hoc ergo propter hoc ' que de retenir quelques accidents ayant suivi plus ou moins rapidement l'immunisation par *la vaccination anticholérique et de lui en attribuer la cause* *Ce n'est* d'ailleurs que dans le recul du temps dans quelques années qu'on pourra juger la methode et apprécier les resultats de l'experience de large envergure tentée cette année en Indo Chine

Les arguments invoqués par les critiques dont nous avons parle tendent a prouver que le choléra a toujours fait son apparition dans le delta du fleuve rouge et dans d'autres régions de l Indo Chine sous l influence de causes que nous ne faisons qu'entrevoir qu'il disparait de lui meme apres avoir fait plus ou moins de victimes quand les pluies surviennent que cela ne justifie pas la campagne anticholérique, semblent émaner d un parti pris systematique et d'un esprit peu scientifique

Le trouble apporté à la quiétude et à la routine des periodes calmes explique cette agitation et cette nervosité

Les arguments invoqués contre l'hostilité des indigenes à nos methodes sont sans valeur et n'ont pas resiste à l examen

Est ce a dire qu'il n'y aurait pas intéret à laisser systematiquement une province isolée à titre de témoin en négligeant intentionnellement de la vacciner pour comparer les chiffres de la morbidité et de la mortalité, je n'engagerai pas de discussion et de critique a ce sujet L'experience serait peut être interessante, mais devant les conclusions fermes de la conference de Paris pouvons nous en conscience la tenter

On a critique aussi les mesures proposées pour l examen des denrées alimentaires prélevees sur les marchés et particulierement du Nuoc Mam des fruits d'ananas debités et exposés en tranches sur l'état des fruits de jaquier des galettes de riz le tout abondamment couvert de mouches la plupart du temps On a même prononcé le mot de mesures arbitraires et peu scientifiques or il nous parait plus logique d'appliquer cette derniere expression à l'omission de cette investigation Nous avons eu en effet l'idée de faire examiner par l Institut Pasteur les echantillons de la faune aquatique dans les eaux des mares au voisinage des foyers de choléra dans les provinces de Phuc Yen et Bach Giang et on a trouvé dans les crabes, crevettes, cyprines du vibrion paracholerique tres voisin du vibrion de Koch mais que l Institut Pasteur n'a pas pû encore indentifier Ces animaux que les indigènes mangent presque crus constituent donc un danger On a trouvé egalement des vibrions dans le nuoc mam condiment que les annamites mangent avec leur riz Il y a donc lieu de s'occuper de l examen des denrées alimentaires comme agent vecteur du vibrion

Nous devons dire en terminant quels ont eté les accidents ou les insucces des vaccinations

Sur 532 milles personnes immunisées de Janvier a Juin 1927

Nous devons a la verité de dire que l'obligation de la vaccination a été generalement admise sans protestation

Dans un rapport du resident de Hadong ce fonctionnaire faisait connaître les resultats heureux obtenus par l obligation de la vaccination imposée aux indigenes frequentant les marchés, les indigenes appartenant aux differentes circonscriptions et se deplacant le plus habituellement dans un but commercial ont pû être ainsi vaccinés, les séances de vaccination a l hopital du chef lieu ont été suivies regulierement Le medecin de Tuyen Quang signale qu il n'y a plus eu de choléra déclaré apres les vaccinations

A Moncay aucun cas de choléra n'a apparu chez les personnes vaccinées et il n'y a pas eu d'accidents dus au vaccin A Soutay aucun sujet vacciné depuis plus de 15 jours n'a eu de choléra Il y a eu quelques cas chez des sujets vaccinés depuis 2 jours jusqu'à 10 jours-ces malades sont tous morts à Cao Bang, les hommes morts dans les villages n'avaient pas été vaccinés Le vaccin n'a pas aggravé la maladie, les injections massives de 3 cc ont été bien tolerés

A Bach Ninh chez 4 sujets des incidents ont été observés sur 51 411 vaccinations Ces accidents se traduisaient sous la forme de lipothymie et ont disparu par absorption de café ou de thé chaud

A Tha Nguyen un seul incident est survenu chez une personne vaccinée

A Hung Jen un linh vacciné en deux séances 2 mois avant a presenté une forme legère de choléra

A Quang Yen chez 8 sujets vaccinés et atteints de cholera il y a eu 3 cas suivis de mort chez des sujets injectés 3 jours 4 jours et 5 jours avant chez les 5 autres vaccinés depuis 7 13 12 et 7 jours avant l apparition de la maladie la guerison est survenue La valeur prophylactique de la vaccination parait tres supérieure à ce que l *on est en droit d en attendre d apres les études faites* au *laboratoire* sur l immunité vaccinale

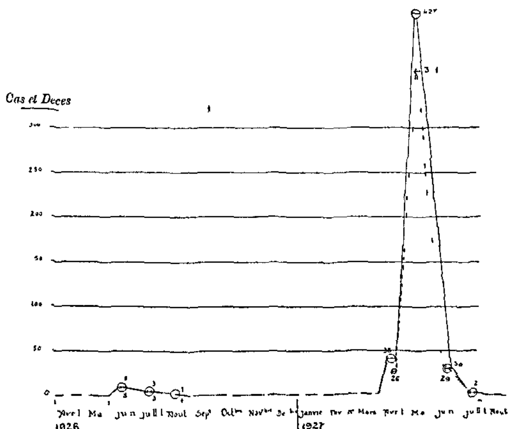
Dans certains postes sur 10 000 personnes vaccinées en milieu epidemique 5 000 n avaient été vaccinés qu une seule fois à 1cc et malgré cela le resultat a été très satisfaisant Nous avons consciencieusement fait état des insucces et des accidents survenus au cours des vaccinations La méthode ne parait pas devoir etre compromise elle a dejà donné des resultats remarquables en Indo Chine Le temps qui est le meilleur des critiques la jugera et nous dira dans quelques années si nous avons été bien avisés de poursuivre avec perseverance la campagne anticholérique par les vaccinations massives à travers les villages du Tonkin dont la densité de la population offrait à nos médecins un champ d activité immense ou s est exercée leur activité et leur dévouement

Tous médecins européens médecins indigenes infirmiers infirmières ont apporté leur contribution à la grande œuvre de la prophylaxie pour arracher à la mort nos populations laborieuses si dignes d intéret et pour sauvegarder le capital que constitue la vie humaine

GRAPHIQUE No 1

REGION DE HONGAY—ÉPIDEMIE DE CHOLFPA 1926 1927

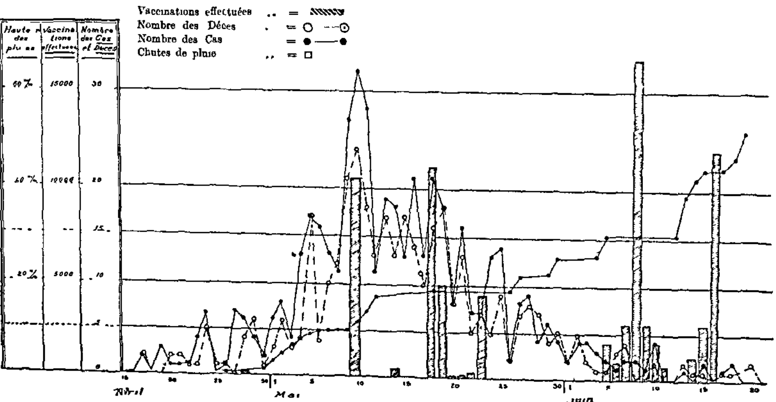
Courbe des cas et Deces de Cholera ẓarınoıs



GRAPHIQUE No. 2.

REGION DE HONGAY—ÉPIDEMIE DE CHOLERA DE 1927.

Courbe journalière des cas et des décès avec indication des vaccinations effectuées et chutes de pluie



Graphique No 3 A

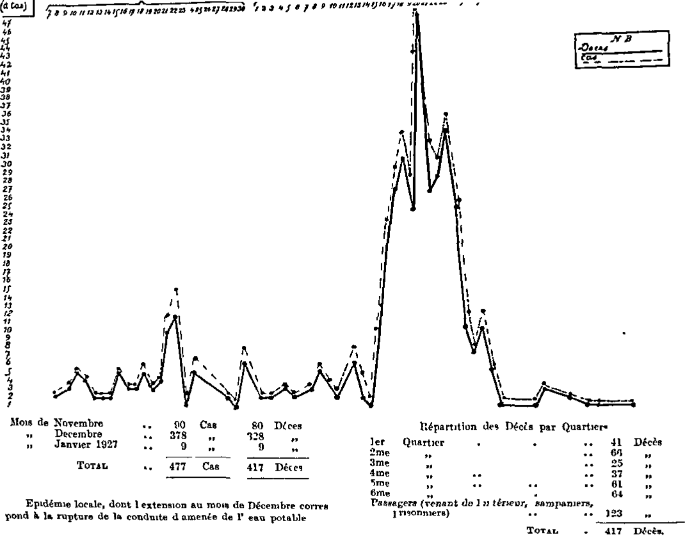
Graphique de l'épidémie de Choléra à Haiphong du 16 Mai au 12 Juillet 1926

Mois de Mai	44	Cas	44	Décès
Juin	262		217	
Juillet	26		26	
TOTAL	332	Cas	287	Décès

Epidémie locale à la suite des inondations qui se sont produites dans tout le Delta

Répartition des Décès par Quartiers

1er Quartier	16	Décès
2me	22	
3me	18	»
4me	19	
5me	78	
6me	78	
Passagers (venant de l'intérieur, sampaniers, prisonniers)	56	
TOTAL	287	Décès



Mois de Novembre	..	90	Cas	80	Déces
„ Decembre	..	378	„	328	„
„ Janvier 1927	..	9	„	9	„
TOTAL	..	477	Cas	417	Déces

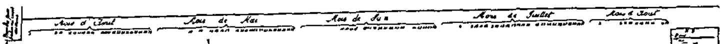
Epidémie locale, dont l extension au mois de Décembre correspond à la rupture de la conduite d amenée de l' eau potable

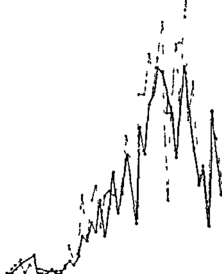
Répartition des Décès par Quartier

1er Quartier	41	Décès
2me „	66	„
3me „	25	„
4me „	37	„
5me „	61	„
6me „	64	„
Passagers (venant de l'intérieur, sampaniers, prisonniers)	123	„
TOTAL	417	Décès.

GRAPHIQUE No 3 C

1927 *Graphique de l'epidémie de Cholera à Haiphong du 3 Avril au 17 Aout, 1927*





		Cas		Décès
Mois d'Avril (du 3)	Cas.	236	Décès	197
Mai		835		748
Juin		60	"	61
Juillet		25		25
Août (au 17 fin)		8		8
TOTAL .	Cas	1 164	Décès	1 039

Répartition des Décès par Sexes

Au dessous de 15 ans garçons	85
Filles	66
Hommes	439
Femmes	449
TOTAL	1 039

Pourcentage professionnel dans ces Deces

95 pour cent environ des décedes Hommes et Femmes exerçaient le métier de coolie

Répartition des Décès par Quartiers

1er Quartier	. 101	Report	464
2me "	.. 159	5me Quartier	333
3me "	. 78	6me ..	124
4me "	.. 126	Passagers	118
TOTAL	464	TOTAL	. 1 039

Mesures prophylactiques

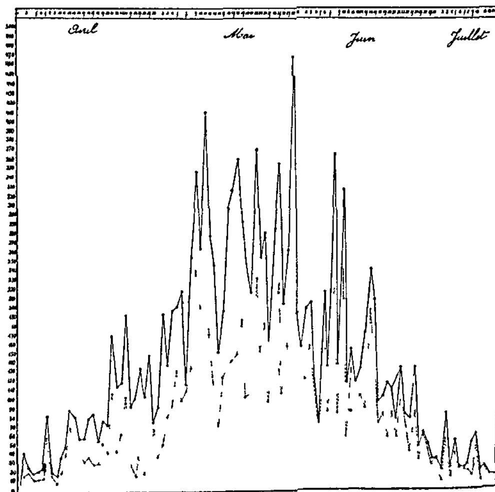
Du 1er Janvier au Aout 1927 le Service d'Hygiene de la Mairie a fait 40 300 Vaccinations anticholériques en séances publiques

Epidemie locale en Avril et Mai *alimentée* à partir du 1er Juin par des provenants de l'Intérieur

GRAPHIQUE No 4

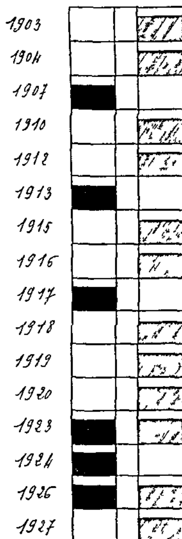
1927. *Courbes du Choléra.*

(Tonkin.) (Indo Chine Française.)



GRAPHIQUE No 5

Annees *Inondations* *Choléra* Province de Nam Dinh



Cette année le Choléra a précédé les inondations

CHOLLRA IN HARDWAR

BY

LIEUT-COL C. L DUNN, C I E, D P H, I M S

AND

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Lucknou.

PRELIMINARY REMARKS

HARDWAR is situated in the Saharanpur district of the Meerut Division of the United Provinces It is here that the mouth of the gorge opens through which the Ganges issues from the Himalayas and enters for the first time upon its journey through the plains of India The river Ganges is the river God of India, it is worshipped and occupies a most important place in the religious life of the Hindus No wonder that Hardwar, where the river God finishes his weary journey in the mountains, is considered a place of extreme sanctity It was in Hardwar according to the Ramayana, when the sixty thousand sons of King Sagar of the great kingdom of Ayodhya disturbed Kapila in his meditations The result was that this large progeny was burnt to death and the ashes lay in a heap at Hardwar When King Sagar heard of this great disaster it was found that the only hope of the children's going up to the heavenly kingdom lay in the coming down of the river Ganges to touch the ashes with the holy water Sagar's wealth and power were of no avail in bringing down the daughter of the Lord of Snow, but one of his descendents, Bhagirath by name, through his religiously devoted life of fasting and austerity, gained the pleasure of Brahma who brought down the river from the heavens and let it loose on the head of Shiva to go down into the plains

This Hardwar then, as old as the Hindu religion, is a small town ' hemmed in to the west by the Sewalik mountains between which and the Ganges the town is situated on land sloping from the mountains to the river, the town filling nearly the whole space available ' The town of Hardwar together with Kankhal and Jwalapur forms the Hardwar Union Municipality of about 31 000 population Hardwar is visited every year by hundreds of thousands of pilgrims from all over India for the purpose of taking a bath in the Ganges The water is considered so sacred that it is taken in vessels specially made for this purpose by the

pilgrims to their homes for pouring on images and giving to the dying Besides these annual fairs, an exceptionally big fair takes place every 12th year and is called a 'Kumbh Fair' The Kumbh occurs at the conjunction of certain constellations, namely, when the planet Jupiter is in Aquarius simultaneously with the Sun being in Aries, which is usually the 13th of April A bath in the Ganges at Hardwar at this time is considered extremely propitious, the concourse of pilgrims, therefore, usually reaches a million or over Cholera has always been the scourge of these pilgrimages The disease has attacked these gatherings for ages with striking persistence Dr C Planck, the first Sanitary Commissioner of the United Provinces, in his interesting report of the Kumbh Fair of 1879 writes —'Very little is known of the history of previous Kumbhs and that little is a history of disease and death' Not only almost every Kumbh has had an outbreak of cholera but many of the ordinary annual pilgrimages have been responsible for the spread of the disease throughout India and beyond it into the continents of Europe and America Of all the cholera disseminating pilgrim centres of India, Hardwar is the most important in so far as the invasion of Europe by this disease is concerned History has shown that the chief epidemic highway of the disease in reaching Europe is the overland route through the Punjab, Afghanistan and Russia It is also known that America has never been attacked direct unless Europe is attacked first The majority of the pilgrims going to Hardwar come from the Punjab and when this, as it were buffer state is itself invaded the disease is more likely to attack Persia, Afghanistan, Russia and finally Europe and America We know that many of the epidemics in India itself and most of the pandemics of Europe have emanated from the pilgrim centre of Hardwar The Kumbh of 1831 was responsible for a severe pandemic that attacked Europe and America and that of 1855 for another similar pandemic, etc

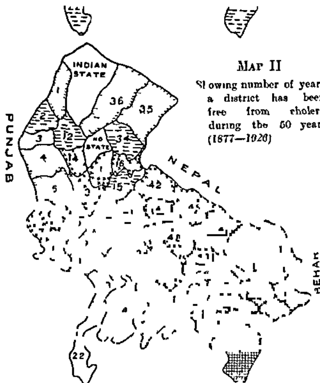
Hardwar is not an Endemic Focus of Cholera

It is on account of the above facts that Hardwar is considered by many as an important endemic focus of cholera, while as a matter of fact it is not Up to date it has never been held that endemic foci of cholera exist in the United Provinces anywhere It is only recently that Sir Leonard Rogers(1) has stated that cholera is endemic in the Gorakhpur, Benares Fyzabad Lucknow and Rohilkhand divisions of the United Provinces—a view with which, however we do not agree But even according to Rogers the Meerut Division where Hardwar is situated is not an endemic area of cholera (*see* Maps I and II) In the Meerut Division Saharanpur is the most healthy district in so far as cholera is concerned Chart I gives the cholera mortality per 1,000 population of each of the 48 districts of the United Provinces being the average for the last 50 years (1877—1926) It will be seen from this diagram that cholera mortality is very low in Saharanpur being 0 21 per mille per annum, and that this district is at the bottom of the scale being the last but one of the total of 48 districts The other districts bordering



MAP I

Showing cholera mortality per 1 000 population in the 48 districts of the United Provinces Average for 50 years (*1877—1926*)



MAP II

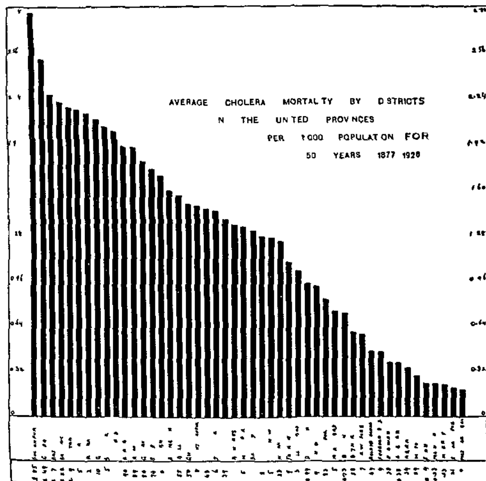
Showing number of years a district has been free from cholera during the 50 years (*1877—1926*)

Division	No.	District
MEERUT	1	Dehra Dun
	2	Saharanpur
	3	Muzaffarnagar
	4	Meerut
	5	Bulandshahr
AGRA	6	Aligarh
	7	Muttra
	8	Agra
	9	Mainpuri
	10	Etah
ROHILKHAND	11	Bareilly
	12	Bijnor
	13	Budaun
	14	Moradabad
	15	Shahjahanpur
	16	Pilibhit
ALLAHABAD	17	Farrukhabad
	18	Etawah
	19	Cawnpore
	20	Fatehpur
	21	Allahabad
JHANSI	22	Jhansi
	23	Jalaun
	24	Hamirpur
	25	Banda
BENARES	26	Benares
	27	Mirzapur
	28	Jaunpur
	29	Ghazipur
	30	Ballia
GORAKHPUR	31	Gorakhpur
	32	Basti
	33	Azamgarh
KUMAUN	34	Naini Tal
	35	Almora
	36	Garhwal
LUCKNOW	37	Lucknow
	38	Unao
	39	Rae Bareli
	40	Sitapur
	41	Hardoi
	42	Kheri
FYZABAD	43	Fyzabad
	44	Gonda
	45	*Bahraich*
	46	Sultanpur
	47	Partabgarh
	48	Bara Banki

	MAP I		MAP II	
Death rate	2 32 to 2 84	▦	*0 to 3 years*	▦
	1 79 to 2 31	××××	4 to 7	××××
	1 26 to 1 78	–	8 to 11	~
	0 73 to 1 25		12 to 15	
	0 20 to 0 73	Blank	16 to 20	Blank
Indian States	*No data*		Indian States	No data

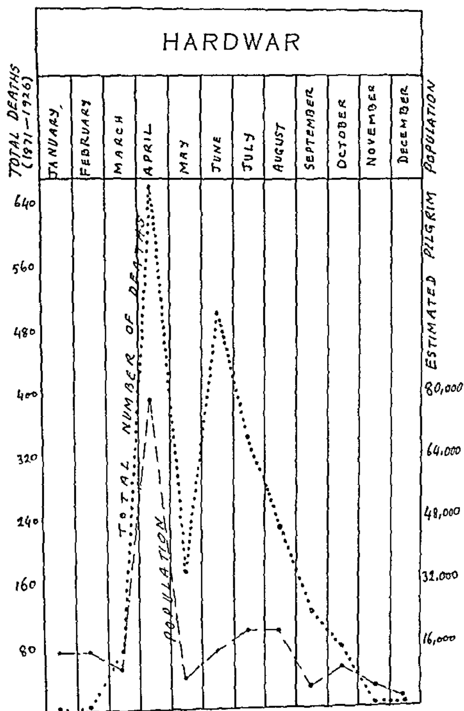
on Saharanpur whether those of the Punjab or the United Provinces are also similarly free from cholera Thus the districts of the United Provinces bordering on Saharanpur namely Muzaffarnagar Bijnor and Dehra Dun are all comparatively free from cholera We know that the part of the United Provinces where Hardwar is situated is an unsuitable ground for this disease

CHART I



The record of the 56 years (1871—1926) for which mortality figures are available shows that cholera disappears from Hardwar during the months of November December January and February (Chart II) There is a steep rise in the month of

CHART II.


HARDWAR
TOTAL DEATHS (1871—1926)
JANUARY
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
POPULATION
640
560
480
400
320
240
160
80
TOTAL NUMBER OF DEATHS
POPULATION
ESTIMATED PILGRIM POPULATION
80,000
64,000
48,000
32,000
16,000

April which is only equalled in its magnitude by the absence of the disease in the preceding months Those who have seen one of these April outbreaks of cholera in Hardwar have been struck by the entire absence of the disease until about the height of the gathering Thus Macpherson writes about the Kumbh of 1783 — It is certain that cholera broke out soon after the commencement of the ceremonies and raged with such fury that in less than eight days it is said to have carried off more than 20 000 victims But so confined was its influence that it did not reach the village of Jwalapur only seven miles distant and ceased immediately upon the concourse breaking up on the last day of the ceremony (2) Again writing about the Kumbh of 1879 Bellow writes — The main point made abundantly clear by all accounts is that the disease did not break out at Hardwar until about the height of the fair which happened to be the middle of the month of April (3) The same is true of the outbreaks of cholera in the Kumbh Fairs that followed If cholera were endemic in Hardwar why then should it be so completely absent until about the height of the gathering? Chart II shows the estimated monthly cholera mortality for the last 50 years The population figures have been arrived at from the estimated number of pilgrims present at all the fairs that take place in Hardwar and are therefore only approximate and may vary within wide limits but the total population itself varies within such wide limits (the highest being thirty times the lowest) that it can accommodate a very wide margin of error It is evident as we all know, that the number of deaths is highest at the time of the highest population It is therefore obvious that the cholera we hear about in Hardwar is really the cholera of the pilgrims and not the cholera of the residents

To illustrate this fact further namely that Hardwar is not an endemic focus of cholera but that the deaths though not so recorded are really mostly among the pilgrims we have prepared Table I This table gives the number of cholera deaths by months in Hardwar Union Municipality for as many years as the records are available (56 years 1871–1926) There are also given for comparison the number of deaths recorded in the rural area of Hardwar The contrast is striking It will be seen that the rural area in the immediate neighbourhood of Hardwar is almost entirely free from cholera for the whole period of 56 years of available records Now if Hardwar were an endemic area of cholera one would not expect such complete freedom from the disease of the villages in the immediate neighbourhood of the town

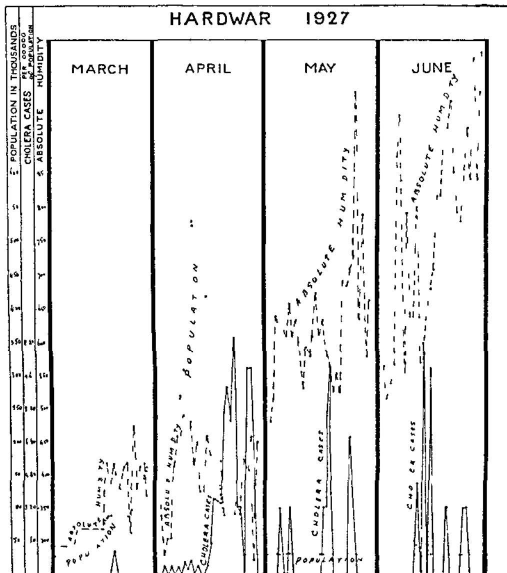
TABLE I—*concld*

Year	HARDWAR UNION MUNICIPALITY												Year	HARDWAR RURAL AREA											
	January	February	March	April	May	June	July	August	September	October	November	December		January	February	March	April	May	June	July	August	September	October	November	December
1910				2	9	5	9	19	4	38			1910												
1911						7	54	15		2	1		1911												
1912	1			2	1			17	7	1			1912												
1913				4	10	50	11	7	1				1913												
1914				2	1	38	[illegible]	5	10	3			1914												
1915			**6**	**186**		**1**	**13**	**1**	**24**				**1915**						**1**						
1916				3	1	23		1	2				1916			1				5					
1917				1		1	30	6		2	2	1	1917												
1918					1	29	33						1918												
1919				56	2	16	9	15	7				1919												
1920				2	10	1	[illegible]	31					1920												
1921				38	3	58	6	16	1				1921												
1922						16			10	3			1922					5							
1923										13			1923												
1924			1	11		36	4	6	2				1924												
1925					8	1			1	1			1925												
1926						5							1926												

ABSOLUTE HUMIDITY AND CHOLERA IN THE KUMBH OF HARDWAR

Chart III gives the daily cholera morbidity for 100,000 population the daily estimated population* and the daily absolute humidity for the four months—March, April, May and June, 1927 Much interest has been aroused

CHART III



* Arrived at for the months of March and April from the number of railway tickets issued.

by the announcement of Sir Leonard Rogers about the relationship of absolute humidity and cholera in his recent paper 'The conditions influencing the incidence and spread of cholera in India'(1) Now Sir Leonard Rogers himself *admits that there is no* relationship between high absolute humidity and incidence of cholera—thus 'Here once more we find no relationship between a high absolute humidity and cholera incidence but when we turn to the months of low absolute humidity we find that in every area in which this reading falls below 0 400 during the cold weather months cholera at the same period falls to a very low rate as in Behar the United Provinces, Central Provinces and North Deccan and altogether disappears as in the Punjab'(1) Now turning to Chart III we find that during the later part of March the absolute humidity was above 0 400 but there was almost complete absence of cholera although quite a large number of pilgrims had already gathered During the month of April from the 5th to 15th the absolute humidity was well above 0 400 so that inasmuch as this factor was concerned there was nothing to prevent a severe epidemic of cholera arising among a pilgrim population which at that time was at its highest While as a matter of fact, there was no cholera at that time From the 16th the absolute humidity began to fall down and remained well below 0 400 until the 25th While that was exactly the period during which the number of cases began to rise up the case rate reaching its highest and remaining high during that period of low absolute humidity It is not necessary further to remark on the relationship between high absolute humidity and the incidence of cholera It will be noticed that the absolute humidity was higher but the cholera case rate was lower during the later part of the month of June than during the month of May As a matter of fact we find that the incidence of cholera in Hardwar depends on the fact that whenever there is an increase in pilgrim population so as to add sufficient pollution to the river it is then that cholera arises

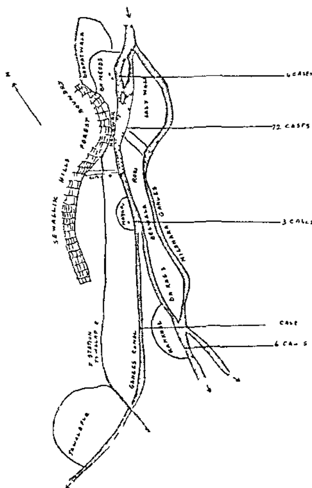
Cholera Cases and Deaths

Up to the end of June 1927, there occurred 99 cases of cholera in Hardwar this year All were confirmed bacteriologically These include six imported cases and also three others that occurred in Raiwala but were *brought to* Hardwar for treatment Of the total, fifty died It should be noted that the first case was imported and so were most of the earlier ones Thus the first third fourth seventh and eighth cases were all imported that is to say they were either taken ill from the trains or developed the disease shortly after reaching Hardwar Only four cases with no death occurred among the residents of Hardwar No case occurred among the residents of the adjoining villages of Jwalapur Kankhal Bhimgoda and Bhopatwala which together with Hardwar make up the Hardwar Union Municipality Of the total of four cases among the residents of Hardwar not one occurred until well after the pilgrimage was over The first two cases among the residents occurred one each on the 20th and 21st April the third on the

18th of May and the fourth on the 25th of June Of the total of 99 cases three occurred in the month of March, 71 in April, 13 in May and 12 in June Of the cases that occurred in April, 44, or 62 per cent occurred during the nine days from the 15th to the 23rd April One thing at least is evident from this, namely, that cholera is not endemic in Hardwar but is an accompaniment of pilgrims How can it be otherwise when we see that there was so complete an absence of cholera in Hardwar before pilgrims came and there were no cases among the

MAP III.

Showing parts of the River Ganges responsible for cases of Cholera.



residents of Hardwar until after the pilgrimage was over ? The bulk of the cases had already occurred among the pilgrims before any occurred among the residents of Hardwar

We have very carefully investigated every case with regard to the source of infection No article of food nor water of any well was found to be the source of infection Out of the 99 cases nine were imported and of the *remaining 90 cases* two were in a moribund condition and could not give any history Out of the 88 cases 59 (60 per cent) used no other water except Ganges water and 27 (30 per cent) used mostly Ganges water In other words 90 per cent of the cases used Ganges water either *mostly or to the exclusion of any other kind of water There were only nine* cases who used Ganges water occasionally but every one of them used Ganges water in exceptionally large quantities one to four days before the attack of the disease If we now turn to Map III we will see that most of these cases used the water of the *Har ki Pairi* pool and the esplanade part of the Ganges *immediately below it* Out of the total of 90 cases two gave an indefinite history and two could not give any history at all Of the remaining 86 cases 55 (64 per cent) drank from the Har ki Pairi and the esplanade part of the river and 17 (20 per cent) drank from the Lalta Rao ghat In other words 84 per cent drank from that part of the river which receives the maximum pollution The whole of the sewage of Hardwar enters this part of the river and it also is the part where the bulk of the bathing takes place Six cases were due to that part of the river receiving the sewage of Kankhal town four cases used the water of the Bhimgoda part of the river three near the canal bridge and one from the canal near Kankhal

Examination of the Sewage

Unfortunately the sewage falling into the river was not examined until the end of May 1927 From the 26th of May up to the 29th of June 173 samples of the sewage of Hardwar were examined and non agglutinating vibrios were isolated from 98 of them During the first *half* of June when the pilgrim *population* increased on account of the Dashera and Nirjala Ekadasi fair (9th 10th and 11th June) 79 per cent of the samples from the sewers showed vibrios On two occasions during this time the vibrios that were isolated from the sewers agglutinated with the anti serum of the cholera vibrio *It is clear that during* this period namely the month of June vibrios were very commonly found in the sewage of Hardwar falling into the Ganges

Ganges Water

Water of the Ganges was daily examined for the presence of vibrios Fourteen places were selected from which samples were daily taken These places are shown in Map IV The pilgrim population during the Kumbh fair were accommodated in the town of Hardwar in Bhimgoda Bhopatwala Rori Island the Bairagi area Kankhal and Mayapur Very few of the pilgrims put up in Jwalapur In other

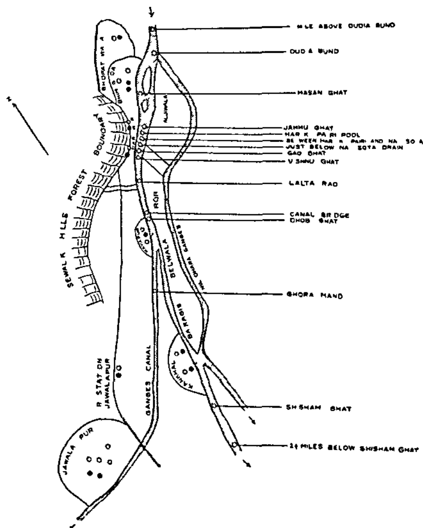
words all the pilgrim population was crowded on the part of the river between Dudhia Bund and Shisham ghat The part of the river between a mile above Dudhia Bund and a mile below Shisham ghat received practically no pollution

MAP IV

SHOWING

WATER COLLECT NG CENTRE o

STOOL COLLECTING CENTRE .... ..●



The part between Jamboo ghat and Lalta Rao ghat received the maximum pollution It is this area especially the Har ki Pairi which is considered most sacred and where most of the bathing takes place It is also this part into which the bulk of the sewage of the town of Hardwar falls

Now if we have a look at Table II it will be seen that the water from the part of the Ganges between Har ki Pairi and Lalta Rao ghat showed vibrios with striking frequency Just below *Nai Sota (Gao ghat) that is to say the place where the main* sewer of the town opens we found that every other sample showed the presence of vibrios It may be pointed out here that most of these vibrios were of the non agglutinating type i e they did not agglutinate with the anti serum of the standard cholera vibrio Apparently it seems that bathing does not add so much pollution (in the way of vibrios) to the river as the sewage of the town And this seems at first sight reasonable to suppose Har ki Pairi pool is the place where an incredibly large amount of bathing takes place and yet the water of this part showed vibrios in 23 per cent of the samples as compared with the water of the Gao ghat (where the main sewers open) showing vibrios in 50 per cent of the samples The explanation perhaps may be the fact that Gao ghat which is a short distance (a few hundred yards) below Har ki Pairi contains in an unmitigated form not only the pollution received at Har ki Pairi but also that super added to it from the opening of the main sewer In that case it will be difficult to decide if Gao ghat received more pollution from the sewage than did the Har ki Pairi pool from the bathing In this connection we have the Bhimgoda pool This is a kind of a bathing pool quite separate from the river Ganges though receiving water from the river It is different from Har ki Pairi pool in that as Har ki Pairi pool is a part of the river and the Bhim goda pool is an isolated tank The amount of water flowing in the Har ki Pairi pool is immensely larger than that in the Bhimgoda pool As to the amount of bathing—the Bhimgoda pool is always packed with bathers—there is not much *difference between the two pools* But only 7 4 per cent of samples of the Bhimgoda pool showed vibrios as compared with 23 1 per cent of the Har ki Pairi pool The Bhimgoda pool does not receive the sewage of the town and the water in it comes from an unpolluted part of the Ganges The Bhimgoda pool was chlorinated but the method was only a rough one (a bag of bleaching powder *in the inlet) and it is probable that the chlorination was not* wholly efficient A similar attempt at chlorination was made at Har ki Pairi also on a few occasions It is very doubtful if this chlorination had any influence in keeping down the *vibrios in the Bhimgoda pool* There was no chlorination after 26th April 1927 Let us take this period of non chlorination from 27th April 1927 to 30th June 1927 and we still find the same difference The Bhimgoda pool showed vibrios in 11 per cent of the samples as compared with 40 per cent *of the Har ki Pairi* pool for that period (27th April 1927 to 30th June 1927) As far as water is concerned it was only the Har ki Pairi pool from which on two occasions a vibrio was isolated which agglutinated with the anti serum of the standard cholera vibrio

TABLE II

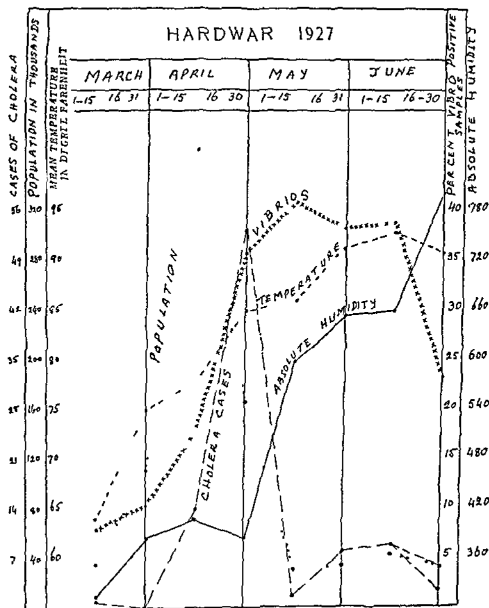
Showing the Result of the Examination of Ganges Water from February to June, 1927 at Hardwar

Serial number	Name of place from where the sample was taken	Total number of samples examined	Number of samples from which vibrios were isolated	Percentage of samples showing vibrios to the total number of samples
1	One mile above Dudhia Bund	7	0	0
2	Dudhia Bund	107	11	10·2
3	Masan ghat	22	3	13·6
4	Har ki Pairi pool	160	37	23·1
5	Between Har ki Pairi and Nai Sota	53	11	20·7
6	Just below opening of Nai Sota drain	68	29	42·7
7	Gao ghat	64	32	50·0
8	Vishnu ghat	49	17	34·7
9	Lalta Rao ghat	107	26	24·3
10	Canal bridge	188	33	17·5
11	Ghora Mandi	13	2	15·3
12	Shisham ghat	151	23	15·2
13	2½ miles below Shisham ghat	27	0	0
14	Nil Dhara	9	0	0
	TOTAL	1025	224	21·8

The percentage of samples showing non agglutinating vibrios varied from time to time This is shown in Chart IV The average of all the places from Har ki Pairi to Lalta Rao ghat has been taken for this purpose There is some increase noticeable from the beginning, e g , the month of March but the highest rise occurs during the month of April and keeps up at this level during the month of May declining to an appreciable degree during the month of June This monthly variation in the vibrionic content of the Ganges water does not appear to have any relationship with the absolute humidity Thus out of the seven fortnightly periods under consideration the two curves proceeded in the opposite directions in three of them At this stage of the investigation there also appears to be no definite relationship between the increase of the vibrios in the water and the number of cases of cholera, although, except for the month of May the two curves show a rather close relation The trend of the curve of mean temperature followed the same

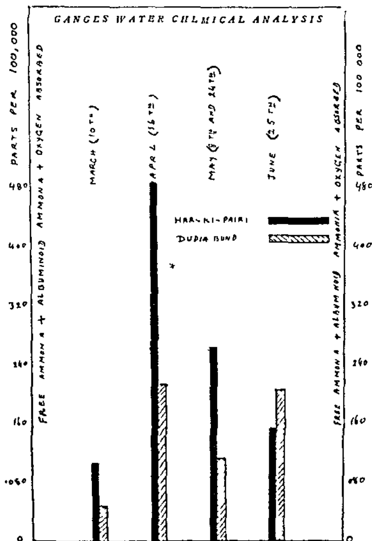
direction as that of the vibrios in six out of the seven fortnights under consideration The increase in the pilgrim population increased the vibrionic content of the water and the vibrios remained at this level for a considerable time afterwards even when the population diminished to very low figures

CHART IV



The chemical examination of the Ganges water was very kindly done for us by Rai Bahadur Dr D D Pandya, D P H (Camb), Assistant Director of Public Health, in the Provincial Hygiene Institute, Lucknow Many samples were examined from several parts of the river and the results of two parts, e g , Dudhia Bund and Har ki Pairi are given in Chart V Dudhia Bund is a part of the river above the town of Hardwar and the pollution here is small as compared with the Har ki Pairi pool which is a part of the river over which the town of Hardwar

CHART V



has sprung up and where most of the bathing takes place This chart gives the sum of the amounts of free ammonia, albuminoid ammonia and 'oxygen absorbed' as a convenient single index of organic pollution It may also be noted that the amount of chlorine, nitrites and nitrates present in the water corroborated the figures given in the diagram Here again we find that the maximum pollution was added during the month of April and this is a sudden rise from the very low level of pollution of the previous month In fact the pollution of even the Har ki Pairi part of the river was so slight during the month of March that the water was actually declared by Dr Pandya as 'potable' During the succeeding three months the water was not chemically fit for drinking purposes The pollution of the water as shown by the chemical examination is in conformity with the vibrionic content except that during the month of May the drop in the vibrios is not so marked as that in the amount of the index of pollution As is to be expected the chemical examination showed much less pollution at Dudhia Bund than in Har ki Pairi much the same finding as arrived at from the vibrionic content of the two parts of the river

It is reasonable to conclude that the non agglutinating vibrios we found in the Ganges water are not among the flora to be found normally in the water On the other hand they come with the pilgrim population and find their way into the river, chiefly in the sewage of the town and also through the bathing Samples of Ganges water taken from parts distant from the sources of pollution were markedly free from vibrios The vibrios added to the river seem to disappear rather rapidly under natural conditions in the river Samples of water taken from the river six to seven miles below the points of maximum pollution were found to be largely free from vibrios in other words, the water had regained normal conditions at least so far as the presence of vibrios is concerned

Varying quantities of Ganges water were taken sterilized and unsterilized, and were experimentally contaminated with small quantities of an emulsion of a 24 hours' agar slope of an agglutinating vibrio At room temperature the vibrios survived in the water for two to three weeks The time of survival was however, longer in sterilized water as compared with unsterilized water In a well water in Hardwar under similar conditions the vibrios survived about a week longer Chemical examination of the water of the well showed much larger amounts of total solids and chlorides than in the Ganges water

WELL WATER

In each of the representative parts of the pilgrim population, e g , Hardwar, Bhimgoda Kankhal and Jwalapur, certain wells were selected as observation wells (*see* Map III) Samples of water from every one of these wells were daily examined besides periodic examination of the water of other wells in the same locality Up to the end of June 1927, 1,481 samples were examined from the observation wells alone (Table III) The wells in Hardwar harboured vibrios less frequently than the Ganges water 6 2 per cent of the samples of the water of

the observation wells showed vibrios as compared with 21 8 per cent of the Ganges water from the part between 2¼ miles above Dudhia Bund and 2½ miles below Shisham ghat In the same locality and during the same time certain wells showed more vibrios than others During the months of March and April all these wells were frequently permanganated so that the remaining period of two months of May and June is too short to give any reliable information as to the seasonal variation of the vibrionic content of the well water Comparing the permanganation period of the two months of March and April with the non permanganation period of the two months of May and June we find that 2 6 per cent of the samples showed vibrios in the former as compared with 10 9 per cent of the latter period (Two wells the examination of which was abandoned after April have been excluded) As to how much of this checking influence on vibrios was due to permanganation and how much to the seasonal variation of temperature etc can only be determined by continuing the observation over the same period next year One thing however, is again evident that even if we take the non permanganation period only, the percentage of samples of well water showing vibrios is much less than that of the Ganges water

TABLE III

Results of the Examination of Well Water in Hardwar from February to end of June 1927

Serial number	Locality where the wells are situated	Total number of samples examined	Number of samples from which vibrios were isolated	Percentage of samples showing vibrios
1	Bhimgoda and Bhopatwala	191	[illegible]	13 1
2	Jwalapur	575	37	6 5
3	Kankhal	229	13	5 6
4	Hardwar	336	16	4 7
5	Mayapur	153	2	1 3
	TOTAL	1 484	93	6 2

Har ki Pairi for comparison = 21 8 per cent.

SOME OBSERVATIONS ON THE BACTERIOLOGY AND EPIDEMIOLOGY OF CHOLERA

BY

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AND

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THE cholera vibrio was discovered by Koch in Egypt in the year 1883 Since that date although the *Vibrio choleræ* (Koch) has been found associated with many epidemics of cholera in various parts of the world extensive search by many competent investigators has failed to find any chronic carrier of this vibrio Nevertheless it was inferred on the analogy of chronic carriers of the *B typhosus* that such carriers must exist and that these carriers serve as reservoirs of infection from one epidemic to another Spontaneous outbreaks of epidemic cholera had also been observed to occur in many places where no history of infection from any outside source could be obtained or be reasonably inferred

It has been frequently assumed that Bengal is the only endemic home of cholera and that every outbreak of cholera in every other country in the world could theoretically be traced to its origin in Bengal

Our researches into the bacteriology of cholera, which were conducted in one *of the endemic areas of Bengal, led us very early to the conclusion that undue atten*tion had been paid by other investigators to the bacteriology of the disease as found at its epidemic height and too little to the atypical forms of the vibrio that had been frequently observed to occur at the beginning and end of epidemics(1) (2)

We therefore set ourselves to discover what becomes of the agglutinating vibrio during inter epidemic periods

In the first place it was observed by us that during the dry hot weather in the Asansol Mining Settlement (March to June) vibrios were very numerous in the ponds or ground tanks' *much frequented by the inhabitants of the settlement for* washing after defæcation The number of vibrios found in such tanks being

approximately proportionate to the number of people using the tanks and inversely proportionate to the volume of water they contained On washing being prohibited in these tanks under the regulations for the prevention and control of cholera in the Asansol Mining Settlement, it was found that vibrios gradually diminished in number and invariably disappeared after 12 to 14 days On washing and bathing being permitted again vibrios constantly reappeared within 24 to 48 hours

'Open bowl' method of cultivation of vibrios

We therefore concluded that the vibrios found by us in these tanks were derived from pollution of the water with human fæces but when we attempted to isolate vibrios from the stools of those frequenting the tanks our efforts were a complete failure

As there could be no reasonable doubt, however that the origin of the vibrios in the tanks was the human intestine we concluded that the ordinary peptone enrichment process used in isolating vibrios from solid stools was unsatisfactory We therefore set ourselves to discover a method modelled on Nature and after much experiment devised what we shall refer to as the 'open bowl' method of cultivating vibrios from stools which was fully described in the *Indian Medical Gazette* of February 1926 and November 1926 The method briefly is as follows

Enamelled bowls of 500 c cs capacity are used each containing 250 c cs of 1 per cent salt solution together with a few c cs of 1 per cent peptone solution

Each whole stool is first thoroughly emulsified in 400 c cs of 1 per cent saline solution and allowed to settle for six hours in a conical glass, 40 to 50 c cs of the clear supernatant fluid being then inseminated into one of the enamelled bowls

For the examination of stools of cholera cases and also as a rule of cholera contacts a different method is used Small quantities of the cholera stools to be examined are first inseminated (by means of dry pieces of wood or twigs from a neighbouring tree) into large test tubes (6 inches by 1 inch) containing 10 to 15 c cs of 1 per cent salt solution To the salt solution in those test tubes we have found that the addition of peptone is unnecessary since 1 per cent salt solution is a selective medium in which vibrios temporarily multiply other fæcal organisms being either held in check or dying out

On the arrival of the test tubes in the laboratory or after two to six hours at room temperature, about six large loopfuls of the surface liquid in the tubes are inseminated into one of the bowls described above

The inoculated bowls in both cases are left in lockers at room temperature, protected from dust and air a few loopfuls of the surface layer of the bowls being tested daily for the presence or absence of vibrios by intensive methods of cultivation through peptone medium and subsequent plating out on bile salt agar Should vibrios not appear in the bowls within one week they are considered to be negative In positive cases vibrios are found in the bowls in two or three days and, when abundant, persist in the bowls up to four weeks

By means of the 'open bowl' method we have been able to prove that in many localities of the endemic area of the Asansol Mining Settlement as many as 33 per cent of the inhabitants are chronic carriers of non agglutinating vibrios

Bacteriological types of clinical cholera cases

With regard to clinical cholera we early ascertained that two bacteriological types existed sporadic cholera and epidemic cholera Sporadic cholera we found in every respect to be identical with epidemic cholera save only in its apparently non infectious or feebly infectious character and in the fact that it is associated with 'non agglutinating vibrios Mackie and Storer(3) however have recorded an outbreak of clinical cholera in a military hospital in Alexandria due to non agglutinating vibrios They also cite the case of a human volunteer who developed severe symptoms of clinical cholera after experimental ingestion of non agglutinating vibrios and our experimental rabbits after intravenous *injection of these vibrios invariably suffer from severe diarrhœa and toxæmia* Epidemic cholera on the other hand is highly infectious and is constantly associated with agglutinating vibrios—sometimes however be it observed of varying degrees of agglutinability It is obvious therefore that agglutinability in a vibrio is not essential for the causation of the symptom complex known as cholera though the communicability of the disease would in the light of our present knowledge seem to be closely associated with this characteristic

In early days when the science of modern serology was still in its infancy Haffkine made the following significant remarks about the cholera vibrio(4)

When the cholera bacillus was first discovered its properties were described with extreme precision which helped in concentrating for a long time all studies on well defined and carefully chosen specimens Little by little as the field of observation grew larger a number of varieties were found with characteristics *differing so largely as to annihilate almost completely the original description* When we open the intestine of deceased cholera patients and investigate the bacteria there the adopted methods will demonstrate the existence of vibrios in which the external forms instead of being the characteristic comma or spirillum will vary between a coccus and a straight thread The number and disposition of cilia the secretion of acids the form of growth in broth will also vary Instead of giving in gelatine a discrete and well-defined figure of liquefaction the variation will extend from the complete loss of this property to a rapid dissolution of the whole medium varieties will be found which grow luxuriantly in given media and others which do not grow there at all some will give the indol reaction and others will lack this property and so on *The first thing to be done is to select* carefully among these the most typical specimens rejecting the others and then to try their pathogenic power When we have done so we shall find such a *divergence in strength that the* extreme forms will not be believed to be the cholera species There will be some commas deprived of any virulence demonstrable on animals and others which will kill the most resistant species

Some will be fatal to a guinea pig in doses of 1/100 of a culture tube, and others harmless in doses 500 times larger'

Regarding the method employed by Pfeiffer for comparing varying strains of the cholera vibrio with the strain selected as 'typical,' he remarks —

'But once such specimens are selected and their particular properties studied they begin to change from the first day they are introduced into the laboratory and no calculation based on these studies is possible In a case quoted by Metchinkoff the proportion of the initial power of the vibrio and the strength it showed at a later trial was as 75 to 1 the vibrio having thus gradually sunk to 1/75 of its initial virulence'

With the advent of the modern method of serological identification it was assumed that all 'true' pathogenic bacteria must retain their specific agglutinability with the type sera whatever other variations they might show but recent observations on the *B dysenteriæ*(5) *B pestis*(6) the spirochætes of relapsing fever(7) and Weil's disease(8) prove that this is very far from being the case

In our efforts to demonstrate the identity of agglutinating and non agglutinating vibrios found in cases of clinical cholera we first attempted to convert the non agglutinating into the agglutinating form by animal experiments

Efforts to demonstrate the identity of agglutinating and non agglutinating vibrios

With this object in view we injected a non agglutinating vibrio intravenously into a rabbit and on its death which occurred unexpectedly after six days we recovered from its gall bladder a partially agglutinating vibrio which was found to be capable of absorbing 80 per cent of the agglutinin from high titre Koch's serum (of the Swiss Serum Institute Berne)

In another instance we made a vaccine of a non agglutinating vibrio obtained from a case of sporadic cholera and injected it intravenously into a human volunteer whose blood showed no agglutinin for Koch's vibrio On this being done the serum of the volunteer was found to be able partially to agglutinate Koch's vibrio (1 20)

In a third and more recent instance by growing for two weeks alternately in bile and broth a non agglutinating vibrio which was derived originally from a case of sporadic cholera we succeeded in raising the agglutinability of the vibrio from 0 up to 1 200 Similar results have also been reported by Toyoshima and Kabashina(9)

Our efforts to convert the non agglutinating into the agglutinating form, while proving that the two vibrios are closely allied serologically were, however, inconclusive and inconstant in results We therefore decided to abandon this line of research and to attempt the conversion of the agglutinating into the non agglutinating form instead

For this purpose a fresh cholera stool, which was subsequently proved in the laboratory to contain great numbers of agglutinating vibrios, was disseminated

into a ground tank, the water of which had been proved by examination to be free of vibrios Samples of the water of the tank in the vicinity of the disseminated stool were then examined every two hours and it was found that the agglutinating vibrios in the cholera stool permanently changed *en masse* into the non agglutinating form under natural conditions in the ground tank after 12 to 14 hours This experiment was repeated on several occasions always with the same result Laboratory cultures of Koch's vibrios were also similarly tested and were found to change into the non agglutinating form after 24 to 36 hours in ground tanks Agglutinability is therefore largely an artificial property developed and fixed by laboratory cultivation since laboratory cultures of agglutinating vibrios take approximately three times as long as the vibrios in the stools from which they are derived to lose their agglutinability under natural conditions in ground tanks

Extended examinations of the stools of epidemic cholera convalescents showed that 80 per cent of these convalescents became chronic carriers of non agglutinating vibrios the agglutinating form permanently disappearing from the stools within two to four weeks

Furthermore it has been a matter of common observation in countries where cholera occurs in epidemic form that during epidemics (due to agglutinating vibrios) non agglutinating vibrios invariably appear in great numbers in polluted water supplies (sewers etc) the non agglutinating vibrios disappearing *pari passu* with the disappearance of the epidemic

Vibrios of varying degrees of agglutinability have also been found by us in eleven cases of epidemic cholera and in two cases we have isolated both non agglutinating and agglutinating vibrios from the same cholera stool

After examination of thousands of stools of healthy persons as well as of survivors of epidemic cholera we have been unable to discover a single permanent carrier of agglutinating vibrios and no authenticated instance of a permanent *carrier of Koch's vibrio has ever been recorded by any other observers elsewhere*

We have therefore been driven to the unavoidable conclusion that the non agglutinating vibrio (which is itself capable of causing clinical cholera) takes on the agglutinating characteristic under certain biochemico physical conditions in the human intestine the nature of which is at present unknown and in this mutation or epidemic form is the cause of epidemic cholera, since it is not unreasonable to assume that a characteristic so unstable may as easily be acquired as lost

Non agglutinating intestinal vibrios, therefore, in our opinion constitute the reservoir of cholera both epidemic and endemic, the degree of non agglutinability in a vibrio apparently depending not only on the nature of its surroundings but also on the period of time which has elapsed since it last existed in agglutinating or epidemic form The nearer to the threshold of agglutinability a non agglutinating vibrio is, the more closely would it seem to be allied both serologically and epidemiologically to the agglutinating vibrio

During the cold weather in the Mining Settlement (November to February) vibrios are so scarce as to be undemonstrable in the water of ground tanks

commonly used by the inhabitants for the double purpose of bathing and drinking, but with the onset of the hot weather (March) they begin to make their appearance and become very numerous as the hot weather advances. It was observed by us that during the hot weather thunder showers always considerably increased the numbers of vibrios demonstrable in tanks. In this connection it is of interest to note that thunder showers during the hot dry weather are popularly credited, in those parts of Bengal where cholera is epidemic during the hot dry season of the year, with the capacity of increasing the intensity of existing cholera epidemics. Chemical analyses of surface washings after thunder showers showed that the percentage of salts as well as of organic matter in such washings is very high. This would reasonably account for the exacerbation of existing epidemics owing to the rapid multiplication of vibrios in infested tanks following the increase of their saline and organic contents. With the establishment of the monsoon, vibrios decrease somewhat in numbers and are even found temporarily to disappear when rain falls continuously for one or more days. During breaks in the monsoon, however, vibrios are always to be found in large numbers in ground tanks.

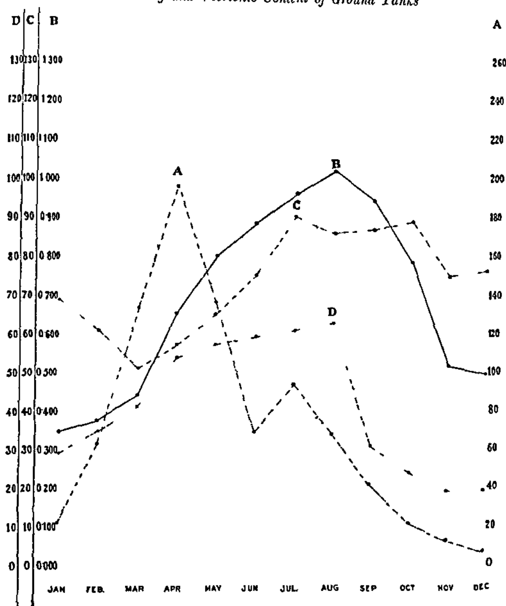
The curve of vibrionic content of water supplies in the Asansol Mining Settlement and the curve of absolute humidity

The curve of vibrionic content of water supplies in the Mining Settlement closely follows the curve of absolute humidity, both curves rising gradually during the months of February, March and April and attaining their maxima during the months of May, June, July and August. The curves then gradually fall during August, September, October and November, reaching their minima during December and January when few or no cases of cholera occur. The annual rise and fall of the number of vibrios in ground tanks, although roughly related to the *epidemic* curve of cholera, is entirely independent of the actual existence of cholera in epidemic form and occurs whether cholera exists or not.

Factors on which the endemicity of cholera in any locality depend

The endemicity of cholera in any locality in our opinion depends primarily upon the existence in the community of great numbers of (healthy) carriers of non-agglutinating vibrios, secondly on the occasional conversion in the intestines of a proportion of these carriers—by some vital process at present not understood—of the non-agglutinating vibrio into its mutation form the agglutinating vibrio, thirdly, upon the widespread and continuous pollution of drinking water supplies (generally surface water supplies, i.e., ground tanks) with the mutation or epidemic form of vibrio through the unhygienic habits and customs

Curve illustrating the Relation of Cholera to Absolute Humidity, Relative Humidity and Vibronic Content of Ground Tanks



Reference —

Cholera Cases (1918—26)	A
Absolute Humidity (1922—26)	B
Relative Humidity (1922—26)	C
Vibronic Content of ground tanks (1926)	D

When once, therefore cholera has been introduced into a community in wide spread epidemic form, great numbers of chronic carriers of non agglutinating

vibrios will remain—apparently for long periods—amongst whom cholera of the sporadic or the epidemic type may occur at any time and if owing to the unhygienic habits and customs of the people, drinking water supplies are habitually contaminated by them then cholera will become endemic in such a locality, provided that the climatic conditions are suitable for the survival and multiplication of vibrios in the drinking water supplies

On the contrary where wholesale pollution of drinking water supplies does not occur, or where conditions are unfavourable to the persistence or multiplication of vibrios in the drinking water supplies cholera cannot become endemic In these circumstances even epidemic outbreaks if such occur cannot become widespread or sustained in character Cholera therefore, in our opinion can only become epidemic in any locality during those periods of the year when, owing to favourable climatic conditions, vibrios are able to persist or multiply in the drinking water supplies of that locality

We also venture to predict that in the deltaic area of Bengal vibrios will be found to persist or multiply in the drinking water supplies of that area at the two periods of the year only when cholera is ordinarily epidemic there one during the hot dry weather immediately before the annual inundation of the country and the other immediately after the inundation has subsided while temperature still remains high and before the onset of the cold weather the flooding of the country during the rains as well as the fall in temperature during the cold weather being both unfavourable to the growth or persistence of vibrios in the drinking water supplies there

On the other hand in the dry and arid regions of north western India the epidemic season of cholera is in general confined to the rains since only during that season is there the necessary amount of surface water as well as the necessary temperature (associated with the insanitary habits of the people) to make an epidemic of cholera possible

Where the percentage of chronic carriers of non agglutinating vibrios remains small, spontaneous outbreaks of cholera will be infrequent and in such areas cholera, if it occurs at all will be chiefly an imported disease

We have been unable to ascertain by experiment whether or not the agglutinating vibrio immediately after it has lost agglutinability is still capable of conveying epidemic cholera but from our combined observations in the field and laboratory we conclude that the vibrio is capable of conveying cholera for some time after agglutinability has been lost and a probable instance of this kind has been recorded by Chalmers and Westerfield(10) A probable factor therefore in the spread of epidemic cholera is the period of time which has elapsed between the contamination of drinking water with the agglutinating vibrio and its ingestion as a non agglutinating vibrio by non immunes

REFERENCES

(1) Rogers, L. (1911) Cholera and its Treatment
(2) Craig (191?) *Ind Jour Med Res*, Vol IV No 4 April, p 6?6.
(3) Mackie and Storer (1918) *Jour Roy Army Med Corps*, August, p 161

(4) MANSON (1911) 'Tropical Diseases' 4th Edn, p 400
(5) CALAIR (1926) *Trop Dis Bull*, January, p 42
(6) *British Medical Journal*, August 7th 1926 p 270
(7) CUNNINGHAM (1925) *Tran Roy Soc Trop Med and Hyg*, Vol XIX, Nos 1 and 2 p 11
(8) UHLENHUTH and HERMANN (1927) *Jour Amer Med Assoc*, 30th July, p 417
(9) 'Studies of Cholera in Japan, League of Nations Health Organization, C H 515—121 P P 1926 Dec, Geneva, summarized in *Trop Dis Bull*, Vol 24, June 1927, p 463
(10) CHALMERS and WESTERFIELD (1918) *Jour Roy Army Med Corps* August p. 161

DISCUSSION

R B Dr C Natesan Moodeliar (Madras) I am thankful to the Congress for having given me an opportunity to listen to such interesting lectures on cholera In the province (Madras) from which I come we are suffering from some disabilities Dr Tomb told us that in his settlement residents were washing themselves in the tanks after defæcating This is a common practice in mofussil areas in the Madras Presidency Villagers wash themselves in a pond or pool or tank after defæcating on or about its bank *In the very tank pool or pond they wash their teeth, they wash their faces, they wash their bodies* They wash their clothes, their cattle and they use the very water for drinking purposes Is it not possible to conserve certain tanks, pools, or ponds for drinking purposes alone ?

Col Dunn placed before us facts that cholera vibrios were found in Ganges water and those among others were the cause of cholera at Hardwar seasons In the province from which I am coming river banks are used as huge latrines Residents generally after defæcating wash themselves in the river and side by side they take water for drinking purposes Should it not be possible to reserve a portion of the river corresponding to the village for drinking purposes and a part further down for washing ?

I am glad that Dr Tomb explained to us the relationship between non agglutinating and agglutinating vibrios In fact the former appears to be the precursor of the latter The appearance of non agglutinating vibrios seems to be the precursor of an epidemic *of cholera I was vacillating as to whether the non agglutinating vibrios should be* recognized or ignored

I have one more point to observe Madras has been subject to periodic attacks of cholera In 1905 we had cholera in an epidemic form unprecedented Every second house had to pay a bill of death In 1914 we had an epidemic This year we were *threatened with an epidemic We had vibrios (non agglutinating) in the water supply* previous to that By the activity of our executive and by disinfection, segregation and anti cholera vaccine inoculations, cholera was brought under control We are obliged to Col Russell our Director of Public Health, who took an active part in the Corporation achieving this object

Col W H C Forster, I M S (Burma) I wish to associate myself with Col Russell's views on the subject of anti cholera inoculation, compulsory or otherwise It was most refreshing to hear such an unequivocal statement which is practically identical in tenor with the views communicated on my behalf to the Health Committee of the League of Nations by Col Graham at the beginning of the year

I agree that the correct method of attacking the cholera problem, the method by which we may expect to minimize and ultimately eradicate the scourge, is the building up of a strong and efficient public health service capable of applying the standard methods of prevention to cholera and all other diseases that is certainly the principle on which we have worked in the Punjab The Punjab, ordinarily, is not troubled by cholera but at times we get severe visitations and it may interest the Congress to know how we dealt with the threatened invasion from Hardwar this year

In the Punjab, in every district, we have whole time fully qualified medical officers of health, and a whole time fully qualified sanitary inspector both borne on the provincial cadre In addition the district medical officers of health have at their disposal a semi permanent staff of medical officers, sanitary inspectors and disinfection gangs trained in anti-epidemic work transferable throughout the province The intelligence department of the district public health agencies is also well organized In 1927 this organization was put to the test with the result that the total cholera mortality for the Punjab Province was under 8 000 as compared with a mortality of 35,000 on the occasion of the last invasion from Hardwar when we had one medical officer of health and no public health organization

Amongst other measures great reliance was placed on the systemic disinfection of rural water supplies and in this respect I can give definite figures In two districts in which the disinfection of water-supplies was almost totally neglected the incidence rate in the infected villages was 1 2 per 1 000 as against a corresponding rate of 0 7 per 1,000 in the case of two districts in which the measure was carefully carried out Potassium permanganate was chiefly used for disinfection but in addition we experimented with a system of chlorine disinfection applicable in the case of our fairs Fairs in the Punjab are a mere bagatelle compared with those of the United Provinces and other parts of India but they play a very important part in the spread of cholera The method adopted was as follows —

A Paterson Pulser Chloronome an instrument which will automatically prepare a solution of chlorine of any desired strength up to saturation, was erected in the neighbourhood of the fair In conjunction with the instrument stoneware capped bottles of 1 gallon capacity were used the Chloronome being set to give a solution of chlorine, one gallon of which would give a dose of 1 5 parts of available chlorine per million gallons to a 100 gallon tank On the fair ground, for the supply of drinking water, portable tanks which take to pieces and can be bolted together again of a unit capacity of 400 gallons were erected according to requirements In disinfection it was only necessary to add the contents of 1 bottle to each tank, the operation being repeated every time the tank was refilled The particular fair selected for trial is a notoriously dangerous one and one which, on last cholera invasion was directly responsible for 800 deaths This year not a single death from cholera occurred directly or indirectly in consequence of the fair The Punjab Government has now sanctioned the extension of the system to every district of the province and has given the money for the erection of a Pulser Chloronome, with ancillary apparatus, at all district headquarters The instrument to which I have referred is on view in the Commercial Exhibition, and being capable of almost infinite variation, it is intended to apply the system to the disinfection of village wells, bottles and a solution being used

to meet the case of a unit capacity of 5,000 gallons, the average water content of a village well

In addition to these special measures the Punjab Government annually spends large sums of money on the improvement of rural water supplies and in time I have no doubt cholera will cease to be a serious menace to the province

Col J D Graham I M S (B India) I would like to take this opportunity of congratulating Lieut Col Russell on the excellent paper he has given us and on the way he has put his back into the work he was asked to undertake I may say that the League has published his paper and it will shortly be available for more general distribution I should also like to congratulate Lieut Col Dunn and Dr Saranjam Khan for their excellent work at Hardwar, and Dr Tomb and Capt Maitra for their work at Asansol

I would like to associate myself with the point made by Col Forster We may inoculate, we may improve water supplies, but the crux of the whole problem of prevention at present centres round the organization of an adequate district health staff Until such a staff, on the line of Col Forster's staff in the Punjab, is organized in every province in India it will be impossible to look forward to an adequate application of rational preventive methods on modern lines against this disease

Lieut Col H C Ross, I M S (Bihar & Orissa) I regret that Col Russell has classified Bihar & Orissa as an epidemic and not an endemic area I have had 23 years' experience in that province and there has never been a year nor even a month in that period when cholera did not exist in some part of the province Bihar & Orissa has a smaller population than Bengal, but a greater average incidence of cholera and I am sorry to say that Bihar & Orissa is the most heavy sufferer of all the provinces from cholera and that it is certainly an endemic area In this connection I invite a reference to a paper on the epidemiology of cholera which I had hoped to submit to this Congress Unfortunately I only returned from long leave in October and it was too late for submission The paper is being published very soon—I hope in the *Indian Journal of Medical Research* *

I do not quite agree with Col Russell as to the importance of rainfall as a factor in the epidemic prevalence of cholera except in so far as rainfall is responsible for humidity, but I entirely agree with his contention that humidity, is a most important factor and that probably relative humidity is the more exact measure of its influence than absolute humidity When Col Russell suggests that humidity is not an important factor in Bengal and perhaps in other deltaic areas I think he has not allowed for the special circumstances whereby these areas are subject to very extensive flooding during the monsoon period when the rainfall and humidity are both at their highest The effects of floods temporarily counteract the influence of humidity which, however, re asserts itself as soon as the floods subside In short the influence of humidity is masked and overborne by the effects of floods but that is a different matter to suggesting that the influence does not act

Col Dunn showed a very interesting lantern slide showing a peak of cholera prevalence at Hardwar in April when rainfall is very low or absent and humidity is low The conditions however, are exceptional and the curve itself is, in my opinion, evidence

* See *Ind Jour Med Res*, Vol XV, No 4, April 1928 pp 951—964 [ED]

that the infection is water-borne which is not usually the case in the seasonal epidemic period

Dr Tomb's experimental work is very interesting, but I suggest that when he puts a pure cholera culture in an open tank or pond and later finds that non agglutinating vibrios are present there is no direct evidence that the vibrios are the same and I suggest that, as it is well known that there are many forms of vibrio resembling cholera, many or most of which are non pathogenic, and none of which is ever directly associated with the existence of epidemic cholera, it is more probable that the cholera vibrios died out and that he recovered other vibrios later which may usually be found in water The assumption that non agglutinating vibrios are a latent form of cholera infection does not seem to be warranted by the known facts that cholera is essentially a human disease and that the only proved source of infection is the human 'carrier

Dr F. d'Herelle (Egypt) In relation to the communication of Dr Tomb, I have to say that I agree with him on the vitality of vibrios in water I have made experiments with waters of the presidency of Bombay, with well waters from the region of Agra, of Lahore, of Kasauli and I have found that, generally, all vibrios were dead within 24 hours, in all samples within 72 hours, either in crude or sterilized water I agree too with the fact that non virulent, non agglutinating vibrios are but a mutation of virulent agglutinating vibrios

What I do not agree with is the possibility of the regression from non agglutinating to agglutinating In our quarantine station of Tor during the last fifty years hundreds of thousands of pilgrims harbouring non agglutinating vibrios in their intestine have passed through the station on their way towards the North, and not a *single* case of cholera has been discovered amongst them nor has an outbreak of cholera ever occurred north of Tor We must conclude that, in Nature, the regression from non agglutinating to agglutinating vibrios does not take place and that carriers of such non agglutinating vibrios are harmless and are never the origin of an outbreak of cholera To say that non agglutinating vibrios may be the cause of the epidemicity is a mere hypothesis, but to show that a Mecca pilgrim carrier of non agglutinating vibrios has never been the cause of an epidemic, that is a fact

Lieut Col C L Dunn (United Provinces) I take exception to one remark of Col Ross that cholera spreads slowly from one district to an adjoining one This was no doubt the rule before the introduction of railways, but now that special pilgrim trains run long distances to places of pilgrimages the situation is changed I can give two concrete examples of this In February 1927, when there had not been a single case of cholera in the United Provinces for over two weeks, a passenger train came from Sealdah station, Calcutta to Muttra with a large number of pilgrims going to a big fair at Brindaban, seven miles from Muttra Several of these pilgrims developed cholera in Muttra and Brindaban, and the result was an epidemic causing 44 deaths in Muttra and Brindaban and no cases anywhere else in the province

Another case occurred amongst passengers arriving in the Jampur district of the United Provinces from Bijapur, Bombay Presidency, about 1 000 miles away, where a severe epidemic of cholera was in progress One died of cholera on the railway platform, the other spread cholera in the adjacent village causing 147 deaths Shortly after this pilgrims went from this district to a big religious fair at Ajodhya near Fyzabad and

in the break up of this fair nearly 6,000 deaths occurred in the adjacent districts These, I affirm, are two examples of the *usual method of the infection of the non* endemic areas of the United Provinces with epidemic cholera

Dr J W Tomb (Bengal) In reply to Col Ross's criticisms, the experiment of converting the agglutinating vibrio into the non agglutinating vibrio in ponds and tanks had all the validity of a scientific experiment On each occasion having selected a suitable tank, guards were placed over it for 14 days to prevent pollution The water was tested daily for vibrios and found negative A cholera stool was then *thrown into the tank and Capt Maitra his* co worker, and *he had* found that thereafter in a period of 12 to 14 days non agglutinating vibrios were to be isolated in fair abundance from the water of the tank They argued therefore, that the origin of these vibrios was the stool which they had thrown into the tank If it was objected that they had thrown in agglutinating vibrios, they, however, answered that this was so, but that examination of the water on many occasions had shown that all these agglutinating vibrios changed into non agglutinating vibrios in from 12 to 14 hours *With regard to Dr d'Herelle's criticisms, it was not accurate to state that cholera* was caused only by agglutinating vibrios Sporadic cholera was always caused by non agglutinating vibrios Capt Maitra and he had always found that in convalescents recovering from epidemic cholera, the agglutinating vibrio regularly changed in 80 per cent of cases into the non agglutinating form in two to three weeks Calalb had found a similar phenomenon in convalescents from bacillary dysentery The agglutinating epidemic form of the vibrio was only a temporary one Any *non agglutinating vibrio in water could theoretically have been an agglutinating* vibrio 12 to 14 hours previously

Col I Froilano de Mello (Portuguese India) Felicite les auteurs des interessants memoires dont quelques unes font un peu table rase des idees que nous avons sur le cholera et son etiologie L'exposé du Dr Tomb est tres important mais il serait a souhaiter que de nouvelles recherches viennent confirmer ses investigations

La prevention du cholera est surtout une affaire d ordre administratif L'orateur *explique pourquoi Goa a ete pratiquement libre du cholera parceque l autorite anglaise* avait fait la notification en du temps

Selon la Convention de Paris les gouvernements provinciaux peuvent faire des accords partiaux pour la notification des maladies Il serait à souhaiter que ce congres advoquat le besoin de tels accords à l Inde entres les diverses provinces pour que la Ligue des Nations put recommander cette mesure preventive aux divers gouvernements et administrations provinciales ici representes

Lieut Col A J H Russell I M S (Madras) In thanking Col Graham for his kind congratulations on my work I would like to say how happy I am in that the discussion has been so vigorous We are, I think, more or less unanimous in this that we cannot accept Sir Leonard Rogers views in connection with absolute humidity and its relation ship to cholera incidence I feel sure that Col Ross and myself are by no means so far apart in our views as he would like us to believe He has indeed specifically stated that a high temperature and high relative humidity with intermittent rains constitute *the favourable* climatic conditions we have been attempting to indicate It is most interesting to note, too, that Dr Tomb has arrived at the same conclusion as we have

in Madras in our statistical analysis, he having reached that conclusion through a purely bacteriological path I can quote examples of the spread of cholera over long distances without intervening cases having occurred similar to those given by Col Dunn and I would refer to the maps shown by me in the Scientific Exhibition which show how a festival centre such as Tirupati can be responsible for widespread infection I show also a map of Tanjore district, part of which is an undoubted endemic centre, where the deltaic area is dotted with numbers of villages constantly infected, in contra-distinction to the non-deltaic area, where very few infected villages occur

As regards organization of a health department being the one method by which, in India, we can hope to combat these recurring cholera epidemics I may add that like Col Forster, we have in Madras a complete health service with a health officer and 10 to 15 health inspectors in each district and this organization which has been developed only within the last six years has already proved its worth in many instances It is, I believe, only by such an organization that we can hope to be successful

We are, I think, also agreed as to the importance of the cholera carrier and future work will have to take this important factor into consideration in all our plans for future preventive campaigns

THE ACTION OF CHOLERA CONVALESCENT SERUM ON COMMA VIBRIOS

BY

A C UKIL M B

Professor of Bacteriology National Medical Institute and Visiting Physician Chittaranjan Hospital Calcutta

AFTER the late Dr P N Das and Dr S C Basu had reported(1) about the marked reduction in mortality of cholera cases treated with convalescent serum it struck us it would be of advantage if we could place the whole subject on a scientific basis

We collected during the course of the current year sera of over 40 cholera convalescents in our cholera ward 30 of which have formed the subject matter of this study All the cases were bacteriologically diagnosed as being due to agglutinating Koch's vibrios

The following sets of experiments were performed —

I AGGLUTINATION (*by the macroscopic method*)

10 sera gave agglutination up to a titre of $\frac{1}{1000}$
9 $\frac{1}{500}$
6 $\frac{1}{100}$
5 did not either agglutinate or gave a doubtful agglutination

Controls kept with anti cholera agglutinative serum gave well marked agglutination in $\frac{1}{1000}$ dilution

II BACTERIOLYSIS *in vitro*

Technique —Two drops of each serum were mixed with 4 drops of vibrionic emulsion 2 drops of 50 per cent alexine and 1 0 c c of physiological saline in a series sterile serological tubes and incubated for 4 hours Controls were kept in tubes without any serum or complement with complement only with convalescent serum only and with normal serum *only At the end of 4 hours a loopful from* each sample was plated on an agar plate and another loopful stained on a slide to show *the disintegration of vibrionic bodies* Readings were taken from the agar plates by counting colonies at the end of 24 hours and 48 hours

Results ,—

18 sera gave a complete dissolution (= no growth of vibrios on plates)
7 ,, ,, ,, partial ,, (= a few colonies on plates)
5 ,, ,, ,, very weak , (= a large number of colonies on plates)

It was found that the 5 sera which gave a doubtful or negative agglutination were those which allowed profuse growth of vibrios on the plates It was further observed that sera giving a good agglutinative titre also showed effective bacteriolysis There is thus a parallelism between the agglutinative and the bacteriolytic titre

III Experiments *in vivo*

The lethal dose, as determined on 6 rabbits, weighing 1 0 to 1 5 kilograms, consecutively, of two freshly isolated strains was found to be 11000 to 12000 millions when given intravenously, causing death in 7 to 8 hours

Five sera giving a good agglutination titre (up to $\frac{1}{1000}$) and 4 sera giving bad agglutination titre (1 in 100) were employed for this experiment While doing these experiments a therapeutic anti toxic cholera serum was received from the Behring-Werke of Marbourg (Germany), prepared under the instructions of Professor Hahn We, therefore included this serum also in our experiments

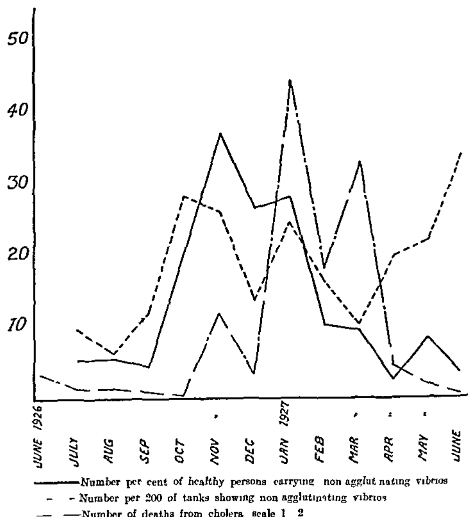
Technique —Single lethal doses were intimately mixed with different dilutions of serum and allowed to remain in laboratory temperature for one hour before being introduced into the veins of rabbits

The results are summarized in the following table —

Sera with good agglutinative titre		Sera with bad agglutinative titre		German anti cholera serum		
Dose of serum	Result	Dose of serum	Result	Dose of serum	Result	Controls without serum
0·01 c c	+7 hours			0·01 c c	+30 hours	+ night
0·05 c c	+41 hours					,,
0·1 c c	+24 hours, one rabbit another survived	0·1 c c	Survived (?)	0·1 c c	+29 hours.	,,
0 25 c c	+27 hours					
0·50 c c	+34 hours one, another survived	0·5 c c	Survived (?)	0·5 c c	Survived	,,
1·0 c c	Uniformly survived	1 0 c c	3+ hours one, another survived			,,

The sign + indicates death after the time interval noted against each.

The vibrios agglutinating with the standard cholera anti serum are, of course, the *Vibrio choleræ* by common consent What are these non agglutinating vibrios ? Could they possibly explain the appearance and dissemination of the agglutinating vibrio during the outbreak of the disease and the disappearance of the latter during the latency of the endemic ?



2 Character of the Non agglutinating Vibrios

They are all motile Morphologically, they are all alike, being uniflagellate and curved in appearance and Gram negative like the typical cholera vibrio Culturally they all grow well in Dunham's peptone water and in the ordinary nutrient agar of pH 7 6 at 37°C , the colonies on the latter are all of a trans parent pale blue colour the culture in the former all give the cholera red

reaction with sulphuric acid We studied the hæmolytic power of 49 of them on sheep's red corpuscles the result was as follows —

Source of the strain	Total examined	Number giving result		
		Positive	Doubtful	Negative
Stools of clinical cholera	26	15	3	8
Stools of healthy persons	10	7	2	1
Water of surface tanks	13	9	1	3
TOTAL	49	31	6	12

It was positive in 63 3 per cent doubtful in 12 2 per cent and negative in 24 5 per cent

It is in their serological character that these vibrios differ markedly from the standard cholera vibrio and from one another

(1) *Reaction with the standard cholera anti serum* —We selected 68 strains of the vibrios for the special study They were from the following sources —

Source	Name of the strain	Total preserved	Number under study
Patients with clinical cholera	Ch_x	28	21
Healthy persons	C_x	21	14
Water from surface tanks	W_x	19	5
TOTAL		68	40

None of these vibrios showed any response to the standard cholera anti serum even at 1 : 10 the titre limit of the standard serum being 1 : 8 000

(2) *Agglutinogenic property* —Up to date we have immunized rabbits with 26 of the strains They all produced anti sera We collected these anti sera after four weekly intravenous injections of the vibrios into the rabbits The following points in connection with these anti sera deserve special notice

(*a*) Their titre limit The titre limit of agglutination of these strains with their own anti sera was very high being the same as that of the typical cholera vibrio to the standard cholera anti serum viz

1 : 16,000 in case of 2 strains
1 : 8,000 ,, 18 ,,
1 : 4,000 ,, ,, 2 ,,
1 : 2,000 ,, ,, 3 ,,

But it was only 1 1 000 in case of four strains and did not rise above 1 200 in case of two more In all these latter cases as will be seen in the table below raising of the anti sera was long deferred

Strain	Date of isolation from the source	Date of collection of serum from the animal	Titre limit of agglutination of the strain with its own anti serum	Interval between isolation of vibrio and raising of the anti serum
Ch_4	23-8 26	2-3-27	1 1 000	Over 6 months
Ch_2	13 9-26	27 8-27	1 1 000	10
C_3	12-8 26	23-8-27	1 1 000	11
C_9	18-8 26	23-8 27	1 1 000	11
C_6	7-8 26	23-8-27	1 200	11
W_2	8 8-26	21 8 27	1 200	, 11 ,

(*b*) Their action on the standard cholera vibrio None of these anti sera had any action on the standard *Vibrio choleræ*

(*c*) Their action on the non agglutinating vibrios

(*i*) The anti sera of two of these strains acted only on the strains producing them viz ,

Name of the strain	Titre of agglutination with the anti serum
Ch_4	1 1 000
Ch_{32}	1 8 000

(*ii*) The other anti sera not only acted on the strains which produced them but also on some more strains to the exclusion of all the other strains with the result that 34 of these strains have already fallen into eight groups all the members of a group agglutinating with the anti sera produced in a rabbit by injection into it of some of the members of the same group and not agglutinating with the anti sera of the other groups

Number of the anti serum =group of the vibrios	Name of the strain producing the anti serum	Number of strains that have fallen into the group			
		Ch	C	W	Total
I	Ch	1	6	2	9
II	Ch_1	2		1	3
III	Ch_8	4			4
IV	Ch_7	6	4		10
V	Ch	1			1*
VI	Ch_{20}	1	1		2
VII	Ch_{34}	2			2
VIII	Ch_4	2	1		3

* Includes W_2 which agglutinates also with the sera of groups I and II

(iii) Four more of the strains reacted to sera of more than one group, viz ,

Strain	Anti sera with which it agglutinated and the titre limit of the reaction				
	I	II	V	VI	VIII
$C_{[illegible]}$		1 : 1,000			1 : 4,000
C_{20}	1 : 4,000			1 : 4,000	
W_2	1 : 1,000	1 : 3,000	1 : [illegible],000		
W_6	1 : 8,000	1 : [illegible],000			1 : 1,000

An attempt at producing anti serum with one of these W's gave after the usual four inoculations into the rabbit a serum the titre limit of agglutination which with the strain was only 1 : 200 The strain had been over 11 months old since its recovery from water before it was used to immunize the animal However this serum weak though it was acted also on members of groups I and II but not on the only member of group V

(3) *Agglutinogenic characteristic of the group members*—In case of two of the groups we could examine agglutinogen of the different members In both these cases the members of the same group were found to produce the same anti serum in the animals immunized with them as will be seen from the tables below —

GROUP III

Particulars of the anti serum				Titre of agglutination with it of			
Strain producing the serum		Date of collection of the serum from the animal	Titre limit of agglutination with it of its own strain				
Name	Date of isolation			Ch_6	Ch	Ch	Ch_{22}
Ch_6	Asansol May 19[illegible]	19-7-[illegible]	1 : 8,000	1 : 8,000	1 : 1,000	1 : 1,000	1 : 2,000
$Ch_{[illegible]}$	[illegible]-8-26	26-11-[illegible]6	1 : 8,000	1 : 1,000	1 : 1,000	1 : 2,000	1 : 4,000
$Ch_{[illegible]}$	[illegible]8-8-[illegible]	19-[illegible]	1 : 8,000	1 : 1,000	1 : 4,000	1 : 8,000	1 : 1,000

Group VII

Particulars of the anti serum				Titre of agglutination with it of	
Strain producing the serum		Date of collection of the serum from the animal	Titre limit of agglutination with it of its own strain	Ch_{4}	Ch_{28}
Name	Date of isolation				
Ch_{4}	28-9-26	12 1 27	1 8 000	1 8,000	1 8 000
Ch_{28}	28-9-26	12-1-27	1 8,000	1 8 000	1 8 000

Vibrios not agglutinating with the standard cholera anti serum on isolation have been known to agglutinate with it after some subcultures (Puttovin, 1913) a non agglutinating vibrio reacted to the standard anti serum to the titre of 1 4 000 after subculture every other day for three months the titre of the serum being 1 10 000 (Flu, 1914) A cholera vibrio divested of the agglutination reaction by passage through water was still found to produce the standard serum *in the animal immunized with it (Stamm, 1914)* This agglutinogenic capacity was believed to be persistent, serving to differentiate the true cholera vibrio from the innocent saprophytes when other characters were lost (Greig 1917) *We see that the non agglutinating vibrios we have been* dealing with —

(*a*) not only did not agglutinate with standard cholera antiserum or with any other heterologous anti sera, and

(*b*) not only did agglutinate with their own homologous anti sera,

(*c*) but produced anti sera (*i*) which had titre limits of agglutination as high as that of the standard cholera anti serum acting on the typical cholera vibrio and (*ii*) which acted in many cases on a number of vibrios to the exclusion of all other vibrios, forming of them so many groups

Therefore if the agglutinogens i e, the substances in the constitution of the vibrios which provoke production of the corresponding agglutinins in the animals under immunization were persistent, we might fairly regard our non agglutinating vibrios as distinct from true cholera vibrios and the serological groups as so many species distinct from each other and from the standard *Vibrio choleræ*

3 Change in Agglutination Reaction

For over six months all the 68 strains continued non agglutinating to the standard cholera anti serum Since then, however, quite a large number of them are showing a change in this respect

(1) *Reaction to standard cholera anti serum* —Forty of these non agglutinating strains, i e, over 58 per cent of them have developed response to the cholera anti serum These changed vibrios include 21 out of the 28 strains from cases of clinical cholera, nine out of the 21 strains from healthy persons and ten out of the 19 strains from water

Source of strain.	Total number under observation	Number which have changed	TITRE OF AGGLUTINATION WITH STANDARD CHOLERA ANTI SERUM TITRE LIMIT, 1 8 000							
			1 4 000	1 2 000	1 1 000	1 500	1 200	1 100	1 50	1 [illegible]
Clinical cholera	28	21	1	3	8	3	[illegible]			4
Healthy persons	21	9		2	4	2			1	
Water of tanks	19	10	1		1		1		2	5

If we ignore the reaction below the titre of 1 200 the proportion of the vibrios which have changed will be —

of the strains from clinical cholera 60 7 per cent
" " healthy persons 38 1
" " water of the tanks 10 5

But C_3 a strain from the stool of a healthy person which appears in the table in the column for the titre 1 1 000 being the limit to which it agglutinated on the 26th July, 1927, began with the titre of 1 20 on the 29th June On the 26th August we found it reacting even at the titre of 1 10 000

The number of members of the different serological groups which have so changed is as follows —

Group	Total number in the group	MEMBERS WHICH HAVE CHANGED			
		Ch_x	C_x	W_x	TOTAL.
I	9		4	1	5
II	3	1		1	2
III	4	2			2
IV	10	6			6
V	1	1			1
VI	2	1			1
VII	-				
VIII	3	1			1
Not yet classified	31	9	5	8	22

The strains Ch_7 and Ch_{11} both isolated from stools of clinical cholera, Ch_7 on the 28th August and Ch_{11} on the 13th September of the year 1926, agglutinated on the 16th May, 1927, with the serum of a cholera patient *at the titre of 1 : 20* These strains were the types of the serological groups IV and II respectively and produced anti sera the titre limits of both of which were 1 : 8 000

(2) *Agglutination with homologous serum*—*In a* number of cases of these changed vibrios the titre limits of the agglutination with their homologous sera were found to have come down Ch_{32} has altogether ceased to react to its own serum and is now agglutinating only with the standard cholera serum at the extreme titre of 1 : 8,000 Isolated on the 27th September, 1926, it continued as non agglutinating up to April 1927, it was found to agglutinate with the standard cholera anti serum on the 25th May to the titre of 1 : 1 000 Then it again began to lose this agglutinability to the standard cholera serum, the titre limit dropping to 1 : 200 on the 15th July, to 1 : 100 on the 22nd July and to 1 : 20 on the 16th August We now grew it in its auto serum, our object was to eliminate from the agglutinogen of this vibrio the receptors which provoked the *production of its own agglutinin in the inoculated* animal and thus to convert it, if possible, into the agglutinogen of the specific cholera vibrio, the result was that after the very first subculture in its own *anti serum its titre to the standard* cholera anti serum reached the limit of 1 : 8,000 and after three more subcultures it lost completely the reaction to its own anti serum

(3) *Change in the agglutinogenic property*—(*a*) Weakening of the capacity of producing group anti serum This has already been noticed in the paragraph on the serological character of the vibrios As has been shown there, the titre limit of agglutination of the serum of the immunized animal after four injections did not rise above 1 : 200 in two cases and reached only 1 : 1,000 in four more

(*b*) Change in the agglutinin produced Not only did the agglutinin produced by the changed strains act weakly on themselves, but in the following three instances it will be seen that their anti sera agglutinated also the typical cholera vibrio

Strain	*Date of* isolation of the vibrio	Date of first notice *of agglutin* ation reaction with cholera serum	Agglutination titre with the *standard cholera* anti serum		Titre limit of agglutination of the anti serum produced by the strain in the rabbit		
			Date	Titre *limit*	Collected from the rabbit on	The *strain*	*Vibrio choleræ*
C_5 .	12-8-26	29-6-27	12-7-27	1 : 1 000	23-8-27	1 : 1 000	1 : 500
W_2 .	8-8-26	11-7-27	28-8-27	1 : 1 000	23-8-27	1 : 200*	1 : 1 000*
W_{16}	23-9-26	1-7-27	9-8-27	1 : 4 000	23 8-27	1 : 2 000	1 : 2 000

* Blood drawn from the rabbit after the third inoculation

4 Change in the typical *Vibrio choleræ*

We learn that Yamanouchi (1921) by cultivating the cholera vibrio in bouillon containing cholera immune serum could remove its agglutinability to it He prepared immune serum with this changed vibrio, and then by cultivating this changed organism again in this auto-serum could restore to it its agglutinability to the cholera anti serum, his work was evidently published in Japanese only We have also been growing the vibrios in the immune sera we find it to be a handy method for eliminating their agglutinability to those sera we are testing the vibrios so changed for their agglutinogenic property By passing intravenously typical *Vibrio choleræ* through a rabbit previously examined for absence of vibrio in the stools and of agglutinin in the blood, we could get from its stool a vibrio which had no reaction to the standard cholera serum including the serum which was produced by itself in the animal and which had reached the titre limit of 1 16 000, in two rabbits that are being immunized with it this non agglutinating variant has produced after four inoculations anti serum which has no action on the standard cholera vibrio including the original unchanged vibrio and which is agglutinating only the variant itself to the titre of 1 4 000

5 Conclusion

We saw that the 68 strains of vibrios we had started with not only did not agglutinate with the standard cholera immune sera but differed from the standard cholera vibrio and among themselves constitutionally they apparently formed species distinct from standard cholera vibrio and from one another We now find that after seven months from their isolation —

(1) Over 58 per cent are agglutinating with the standard cholera anti serum two to the extreme titre of 1 8 000 and 1 16 000 respectively

(2) One has lost the agglutination reaction with its homologous serum on being cultivated in it and is agglutinating with the standard cholera serum only at the extreme titre of 1 8 000

(3) Three are producing in rabbits under immunization with them sera which are also agglutinating the typical cholera vibrio

They are in fact in all stages of transformation from the non-agglutinating forms to the state of the typical cholera vibrio We have also seen that the typical cholera vibrio passing through an immunized animal appears in the stool as a non agglutinating vibrio i e a vibrio having no reaction with the standard cholera serum and that this variant produces in rabbits immunized with it agglutinin acting on itself but without action on the original strain of any other typical cholera vibrio Therefore we may fairly infer —

(1) That over 58 per cent of these changed agglutinating vibrios are nothing but vibrio of cholera,

(2) That they have undergone alteration in the agglutinogenic constitution, and

(3) That they are capable of reversion into their original agglutinating type

The strains Ch_7 and Ch_{11} both isolated from stools of clinical cholera, Cl
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W_4	8-8-26	11-7-27	28-8-27	1 1 000	23 8-27	1 200*	1 1 000
W_{16}	23 9 26	1-7-27	9-8 27	1 4 000	23-8-27	1 2 000	1 2 000

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REFERENCES

PUTTOVIN (1913)	*Bull de l office Internat d'Hyg Publique*, Vol V p *1163*
FLU (1914)	*Trop Dis Bull*, Vol VI, p 39
STAMM (1914)	*Ztet u Hyg*, Vol LXXVI, p 469
GREIG (1917)	*Ind Jour Med Res*, Vol IV, p 659
YAMANOUCHI (1921)	'Studies of cholera in Japan published by League of Nations, p 29

DISCUSSION

Lieut Col W C Ross I M S (Bihar & Orissa) In considering Dr Brahmachari's paper there are two possible fallacies in the work which appear to me to be of great importance Dr Brahmachari infected a rabbit with pure cholera and immunized it to such a degree that its serum had a titre of 1 16 000 He found the cholera vibrio in the rabbit Later he found a non agglutinating vibrio which he suggests is a transmuted form of cholera vibrio I would suggest that it was always possible that the food and water given to the rabbit may easily have infected it with a second infection of non agglutinating vibrios especially when we know that these are prevalent in the water supplies It is not a justifiable assumption that they must be the same and that the cholera vibrio has assumed non agglutinating properties Further when he refers to a series of agglutination tests in which the titre first rose to a high figure and then fell off again, I would suggest the more obvious explanation of the presence of a bacteriophage rather than that the cholera vibrio had twice changed its capacity for specific agglutination

With reference to the general discussion on the theory that cholera vibrios may be thus variable in specific agglutination tests and may live in a latent form in the water supplies I would suggest that it is not reasonable to contravert fundamental bacteriological principles governing specific reactions in order to explain the presence and activities of non agglutinating vibrios We have the classical and historical example of the Widal reaction for typhoid fever which led to a storm of contentious argument for many years The reaction is, and always was, specific but in a small percentage of cases it failed The eventual solution of that problem was the discovery of *B paratyphoid* A and B It is by analogy equally possible that non agglutinating vibrios may be pathogenic and may cause disease in rabbits and perhaps in human beings but it is certain that Asiatic cholera is a specific bacteriological entity with a specific reaction and that the cholera vibrio is the cause of epidemic cholera and almost certainly the sole cause Other vibrios may produce pathological symptoms but they are incapable of producing epidemic cholera I think it is much more probable that the non agglutinating vibrios found in the water supplies and in the human intestine in Bengal constitute a separate bacteriological entity and, though they may be pathogenic, yet they are not transmuted cholera vibrios and are not the cause of epidemic cholera

Dr F d Herelle (Egypt) In relation to the hæmolytic power of the vibrios, I had the opportunity to test in India about three hundred strains of agglutinating vibrios recently isolated from the stools of acute cases I have used human blood, for the reason that man is the only being sensible to cholera with not a single exception,

the three hundred vibrios tested were all hæmolytic, most of them *strongly* hæmolytic

Dr C G Pandit (Madras) I Non agglutinating vibrios from water supplies have been subcultured for over two years with no change in their agglutinating characters

II I should like to inquire if Dr Brahmachari s culture was pure as regards the smooth and rough types of colonies, as these, as recent work suggests, modify greatly the agglutinating characters

Dr J C Mukerjee (Bengal) Pointing out that he had worked in the cholera inquiry with Col Greig, I M S, from 1912 to 1916 said that in a good percentage of acute cholera cases both agglutinating and non agglutinating vibrios were found The sera of the patients from whose stools these vibrios were isolated agglutinated only with Koch's cholera vibrio but never with the non agglutinating vibrio This proves that immune body was developed only against the true cholera or agglutinating vibrio but not against the cholera like vibrio Experiments in connection with the transmutation of one species of vibrio into another proved most unsuccessful So high a transmutation from one species of vibrio to another as 58 per cent, within seven months appears to be strange and requires confirmation by others before it can be accepted

With regard to Dr Ukil s paper on the action of serum of cholera convalescents on the cholera vibrio it has been found that agglutinins (anti bodies) are developed as early as the third day to a very high titre in acute cholera cases who show *rapid convalescence* Those cases which showed no agglutinins or very slight agglutinins in their sera against the cholera vibrio ended fatally So far, the efficacy of anti cholera serum from animals in treatment was doubtful but if the sera of convalescent cholera cases appear to be beneficial in curing cholera cases when given early the method would be worth trying How such a small quantity of serum worked in staving off complications and lowering mortality had yet to be investigated

Dr E P Hicks (Shanghai) It would be interesting to hear something of the reactions of non agglutinating vibrios other than the serological such as the production of cholera red, hæmolysis sugar reactions etc In the diagnosis of cholera I have often isolated vibrios which do not agglutinate with specific cholera serum Some of these become agglutinable after a few days subculture, and these give the usual reactions Others do not become agglutinable and these nearly always give abnormal reactions they may or may not form cholera red they may produce hæmolysis, and they may show variation in sugar reactions especially in failing to form acid from saccharose I think these are points which should be considered

Dr Saramjam Khan (United Provinces) The strains tested by Dr Brahmachari were mostly from clinical cholera cases, and it is a known fact that strains recently isolated do not agglutinate but do so later Was there any standard method of agglutination used because the time, temperature and personal factor are things to be taken into consideration? What precautions were taken to ensure the purity of cultures?

It would have been more interesting had Dr Brahmachari given us the percentage of changed strains from the non agglutinating to the agglutinating form for the strains isolated from water

Capt G C Maitra, I M S (Bengal) The role that non agglutinating vibrios play in the production of clinical cholera was first investigated by Greig in India from 1912 to 1916 I had the honour of being associated with him from the beginning to the end of his enquiry and I can say from personal experience that these atypical vibrios bear as much ætiological significance as the typical *Vibrio choleræ* of Koch does in the causation of this disease This was further verified by me personally when I subsequently had the opportunity of doing it myself in my own way Greig tried to classify these vibrios serologically by agglutination and absorption tests The result was that he was able to classify only 65 out of 78 strains which he studied Those that were classified fell into six groups The unclassified strains remained each a member of a group by itself Thus it might be seen that there would be no end of serological groupings and sub groupings if one tried to classify them in this way These vibrios with which we are dealing in Bengal however, do not differ from the typical Koch's vibrio in broad features They are all comma shaped motile monociliate indol formers and liquefy gelatin in the usual way As a rule, they are non toxic to pigeons but lethal to guinea pigs and rabbits So far they agree with Koch's vibrio The relationship which they bear to the epidemiology of the disease was not investigated by Greig whose work was interrupted by the War

When I took up the thread of his enquiry in 1923 and started investigations with Dr Tomb in the rural areas of the Bengal coalfields amongst the permanent residents there it at once became evident that the bacteriology of a sporadic cholera case was quite different from that of the disease at its epidemic height Early cases in the epidemic season and all sporadic cases in the inter epidemic season were, as a rule found to be associated with non agglutinating vibrios The agglutinating vibrio (Koch's type) is found only when there is an epidemic either of spontaneous origin or imported from outside I also noted that when the epidemic subsided non agglutinating vibrios were isolated from an increasing number of cases either alone or in conjunction with the agglutinating vibrio Finally the latter disappear altogether from the field leaving the non agglutinating vibrios to keep up the case incidence in the endemic area during the quiescent period between two epidemic seasons This cycle of events is repeated from year to year From this I and Dr Tomb concluded that the agglutinating vibrio is the epidemic vibrio while the non agglutinating vibrio is the cause of sporadic cholera When we started investigating the source of these two types among supposed carriers, we found that about one third of the population of the endemic area were carriers of non agglutinating vibrios but no permanent carrier of the agglutinating vibrio was to be found anywhere Even survivors of epidemic cholera clear themselves of Koch s type of vibrio in about three to four weeks time and, if they become chronic carriers at all they carry non agglutinating vibrios From this Dr Tomb and myself concluded that agglutinating vibrios change their serological characters in carriers and persist as non agglutinating vibrios and that these latter serve as the natural reservoir of cholera both endemic and epidemic and that this is so has been verified by various observations under natural and artificial conditions which have been broadly outlined in our joint paper read by Dr Tomb

Whether a non agglutinating vibrio can be converted into a fully fledged agglutinating vibrio experimentally is still under investigation Although we have been

partially successful in this line and have put up specimens in the exhibition, our results are still inconclusive But there are ample grounds for believing that this happens under natural conditions and thus precipitates an epidemic of 'spontaneous origin About two years ago we investigated an epidemic in an isolated hamlet in the Asansol mining settlement where a boy of nine years developed clinical cholera and was nursed by his mother The boy survived and a non agglutinating vibrio was isolated from his stool About the fifth day of the boy s illness his mother developed the disease and Koch's type of vibrio was recovered from her stool A few more cases occurred among close neighbours and there was a small localized epidemic consisting of 10 or 12 cases, in all Koch's vibrio being isolated from all the subsequent cases

In another instance a cholera epidemic broke out in a distant village about six miles away from the nearest railway station The first case occurred in a Mahommedan house after a religious feast in which the patient participated although he had been suffering from chronic diarrhœa for a long time

He subsequently developed cholera and died His stools could not be examined directly, but the washings of his soiled bed linen gave a profuse growth of non agglutinating vibrios Agglutinating vibrios were isolated from all the subsequent cases in the same and neighbouring houses From these two instances it would appear that non agglutinating vibrios take up agglutinating characters after successive passage through non immunes

The sum total of all these observations is that the serological character of a vibrio is by no means immutable and that the agglutinating vibrio becomes non agglutinable and vice versa As the latter is widely distributed in nature, one is justified in holding that these non agglutinating vibrios constitute the natural reservoir of cholera both endemic and epidemic

Dr B B Brahmachari (Bengal) replied (1) As to the suggestion that the conversion of non agglutinating vibrios into agglutinating vibrios might be due to contamination, I might tell you as is well known to Dr Tomb and Capt Maitra, that I was strongly biased the other way for it was inconceivable to me that vibrios so different from one another serologically could be mere modifications of the same typical cholera vibrio, and, that when after seven months I noticed that some of my strains were agglutinating with the specific cholera anti serum I was taken by surprise and my assistants could hardly believe their own eyes I can assure you that every precaution was taken against contamination that change in so many strains could be due to contamination is out of the question

(2) As to the technique of our agglutination test it was the ordinary capillary tube method of sero sedimentation the temperature being that of the incubator for two hours and of the ice chest for the remaining 22 hours though we now find treatment for at most two hours is enough for all practical purposes

(3) As to the query if the vibrios which changed were all from cholera cases, I have already shown in my paper that 21 of the changed non agglutinating vibrios were out of 28 strains from clinical cholera, nine were out of 21 strains from healthy persons and ten were out of 19 strains from water

(4) Regarding the transformation of the agglutinating vibrio in to the non agglutinating form by passage through a rabbit the suggestion of Col Ross is that the rabbit

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might have been carrying the non agglutinating vibrio at the start We took care to examine 15 rabbits particularly for vibrios in the stools, they were all found, as usual free from them then one out of these 15 rabbits was taken the examination of its stools was repeated for some weeks and finally its blood was tested for agglutinin, I can assure you, therefore, that the rabbit did not carry vibrios from the beginning As to the strain itself used for the experiment, we got it from the stool of a clinical case of cholera it agglutinated with our own cholera anti serum as well as with that from Kasauli, to the titre limit of 1 8,000 and also with the serum of a patient convalescent from epidemic cholera to the titre of 1 400 To the suggestion of Col Ross that the rabbit might subsequently have got infected with the non agglutinating vibrio and to the assertion of Dr d'Herelle that such a change is impossible, I would say, if permitted to go beyond the scope of my paper, that, since writing it, we have reconverted this non agglutinating vibrio into its former agglutinating form On successive passage through non immune guinea pigs it began to agglutinate with the specific cholera serum till the titre rose to 1 4 000 the reversion by passage through guinea pigs stopped at this stage and was found to be still incomplete We then grew it with the anti serum of its non agglutinating stage with the result that the change became complete and it was once more the typical cholera vibrio agglutinating with specific cholera anti serum to a titre limit of 1 8 000 and producing the specific cholera anti serum with a titre limit of 1 16 000 As to the agglutinating vibrio losing its agglutination reaction on account of the action of such factors as bacteriophage in the intestine of the rabbit as suggested by Col Ross, I would say that the agglutinating vibrio not only lost its agglutination reaction with cholera serum but acquired the property of producing in animals an agglutinin of its own

(5) As to strains kept for three years and still showing no change in agglutination reaction, the number of strains must have been few, besides, we find that frequency of subculturing expedites the change though we do not know definitely as yet the relative importance of the two factors the age of the strain and the frequency of the sub culture, in bringing about the change

(6) As to the presence of non agglutinating vibrios in water having no connection with outbreaks of cholera as urged by Col Russell I have shown in the graph in my paper that the curve of non agglutinating vibrios moved with that of the mortality from cholera shooting up to its peak in November, then coming down slightly in December and January and finally dropping through February, March and April to its trough for the rest of the year

DYSENTERY, SPRUE AND INTESTINAL INFECTIONS

THE DYSENTERIES IN BENGAL

BY

A C UKIL

Professor of Bacteriology National Medical Institute and Visiting Physician Chittaranjan Hospital Calcutta

WEDNESDAY DEC 7TH 10 A M TO 1 P M

THE dysenteries take a toll of nearly 1 30 000 deaths (out of a total population of 4½ crores*) every year whereas cholera takes a toll of 80 000 a year One fifth of the total number of deaths in Calcutta are due to dysenteries whereas cholera carries away half that number Nearly a century ago Norman Chevers recorded that three quarters of the total deaths amongst the lower orders of Indians were due to diarrhoea They are a constant and heavy drain on the population of this country, but not being spectacular in outbreak though far reaching in effects they have not received the measure of attention they deserve from the public health authorities

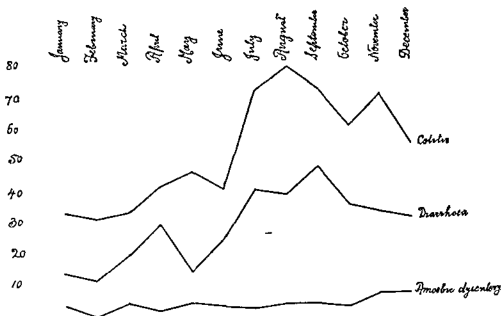
EPIDEMIOLOGY

Bengal is made up of a low lying tract of alluvial soil interspersed with rivers and badly drained sheets of water The lower part consists of the deltaic area from two of the biggest rivers of India The rainfall is abundant the temperature equable and the humidity high at certain periods of the year The rainfall begins to rise in June and is usually greatest in July August The mean temperature curve begins to fall with the onset of the rains

Cases of dysentery are lowest during the driest earlier part of the year—January to April A sort of parallelism has been noticed between the onset of the monsoon and the rise in dysentery and diarrhœa cases—cases begin to increase with the onset of the rains and reach the maximum usually in August September This rise is sometimes continued to December after which the curve invariably falls This has been found to be the case not only in rural areas where there is no control of water supplies and where water is easily

* 1 crore—10 000 000

contaminated by surface washings during the rains but also in cities and towns having a filtered water supply and in jails where water supplies are carefully controlled and periodically examined by the Public Health Department Rarely, an outbreak occurs in winter These outbreaks of dysentery and diarrhœa are more common in the eastern part of Bengal which contains more rivers and water logged areas than other parts of this presidency We will illustrate the incidence by data in one of the jails (Midnapore Central Jail) from an average of 4 years' statistics



Dysentery and diarrhœa cases in the Midnapore Central Jail from 1924 to 1927

Remarks Clinical cases of bacillary dysentery have been entered in the above chart under the heading 'Colitis' Many of the 'Diarrhœa' cases have been shown to be due to a chronic bacillary infection

In a year of heavy monsoon, there is a corresponding rise in dysentery cases The incidence of diarrhœas in Calcutta follows a closely parallel curve, being increased during the rainy season and autumn There is reason to believe, as will be shown later, that most of these cases of diarrhœa are caused by a mild and chronic bacillary infection

The monsoon outbreaks are, however, of mild virulence, and never assume epidemic proportions, as with the more toxic form of the disease which occurs less commonly Three types of cases occur– acute, sub acute and chronic Sub acute and chronic cases are far more common than acute cases As has been shown by Cunningham(1) chronic cases as well as many of the diarrhœic forms are due to a recrudescence of the original infection

Mortality and morbidity

The relative incidence of dysentery and diarrhœa in jails may be taken as representative of the prevalence in outside population, as their periodicity and [illegible] more reliable data

[illegible] er cent

[illegible] 43 per cent are admitted for diarrhœa of which 0 02 per cent die The relation of mortality to morbidity thus stands as 1 34 Thus if there are 130 000 deaths in Bengal annually from dysentery, 4 420 000 people (or roughly *one tenth of the population of Bengal*) must have suffered from it for a certain part of the year This is perhaps an under estimate as the sanitary con ditions regarding food water supply and cleanliness are much better in the *jails than among the civil population The economic loss consequent upon* incapacity for work and invalidity must be enormous to the nation

Diet and disease

Rice is the staple diet of the people in Bengal Madras and Burma But bowel disorders of the nature of dysentery and diarrhœa are remarkably rare in Madras and Burma Next comes the excess of *leafy* vegetables taken by the people They are no doubt consumed in excess during the monsoon months when they are abundant They frequently set up mild irritations of the bowel, *but they are hardly likely to cause an infection*

Age sex and nationality

As regards age, sex and nationality (Hindus and Mohammedans) there is no marked variation, except that old people seem to be slightly more prone to them Children's cases have been too few in our series to enable us to form an opinion

Bacteriology

Bacillary and amœbic forms are the prevailing types of dysentery in Bengal, the former being the commoner comprising 5 to 6 times or more the number of cases of amœbic dysentery Cunningham and King(2) found in 1916 17, among a jail population of 3 460 distribution of the dysenteries as follows —

Bacillary	57 32	per cent
Amœbic	5 10	,,
Both combined	3 82	,,

Search for causative organisms became negative in 33 76 per cent of cases with mucus in stools

Acton and Knowles(3) writing in 1924 considered bacillary dysentery to be 5 to 6 times as frequent as amœbic dysentery In our series of consecutive 1,500 stool examinations, spread over a period of three years and half, we found

the incidence of amœbic dysentery to be 33 per cent or one third of that of bacillary dysentery Some of the statistical data might be interesting

I	Stools containing both mucus and blood	316
II	pus cells and mucus only but no amœbæ	364
III	pus cells but no mucus	90
	Total	770
	Less cases showing vegetative and cystic *Ent hystolytica*	260
	Balance	510

Among these 510 samples Shiga's bacillus was isolated in 44 cases [this includes 22 strains isolated during an outbreak of Shiga dysentery in Calcutta in 1924(4)] *B flexner* was isolated in 64 cases and other Gram negative non lactose fermenting bacilli not belonging to the stable Shiga and Flexner types in 150 cases In the remaining 256 cases no incriminating cultural organisms could be detected We have included the cases showing mucus and pus and those showing pus cells only in the category of bacillary dysentery because of the evidence adduced by Cunningham and King (*loc cit*) *regarding the ætiology of such cases* If we leave aside the 22 cases isolated during an epidemic in Calcutta the proportion of mannite fermenters to non mannite fermenters comes up to 61 3 per cent 38 7 per cent

We confess we have not been able to observe and follow each case so closely as Cunningham and King did in the Eastern Bengal jails We received the samples from Calcutta and its neighbourhood within a couple of hours after arriving usually much earlier A record was kept of the day of illness of the patient and the stools were plated on McConkey s bile salt lactose agar plates by a very reliable method (modified Whitehead and Kirkpatrick method—(*loc cit*) Two or three colonies were fished out next day from this plate and subcultured on lactose litmus agar plates to purify and verify that they did not ferment lactose They were then subcultured on agar tubes for fermentation and other tests

In an earlier paper myself and Dr A K Sen(5) gave the results of a study of 60 strains of non lactose fermenters from the stools having the typical characters of *acute bacillary dysentery alkaline reaction characteristic cellular exudate* paucity of bacteria and naked eye appearance All the strains fermented glucose with gas production however Each strain was tested for motility staining peculiarities sugar reactions with lactose litmus milk glucose mannite maltose saccharose dulcite xylose salicin inosite raffinose arabinose adonite and inulin, fluorescence and fragmentation of neutral red agar and blackening of lead acetate Voges and Proskauer reaction and indol production and sero agglutination with *B paratyphosus* A *paratyphosus* B and *B enteritidis* Gaertner high titre serum

Out of these 60 strains 36 strains were found to be permanent non lactose fermenters and the remainder late lactose fermenters fermenting it in 1 to 3 weeks

Only three of the strains agglutinated with *B enteritidis* Gaertner serum and one with *B paratyphosus* B serum the remainder did not agglutinate with either of these sera Twenty one of the strains did not produce indol

Seventy five per cent of these non lactose fermenters proved pathogenic for rabbits when given intravenously in doses of 0 25 c c to 1 0 c c of a 24 hours' agar culture and containing 4 000 million organisms to the cubic centimetre

At one time we used to think that they were association organisms found in a dysentery case after the first three days of illness as has been pointed out by Manson Bahr Perry and Manson(6) But their detection in quite early stages of the disease (within the first 24 hours) and during short epidemic outbreaks leads us to think that they play an important role in the causation of bacillary dysentery in Bengal especially in view of the fact that they are pathogenic for laboratory animals that they agglutinate sometimes with the patient s serum after recovery (this has been done in a small number of cases) and that vaccino therapy with these strains often yields successful results

As regards grouping of these bacilli they must be labelled as pseudo dysentery bacilli belonging to the paratyphoid enteritidis group In addition to the stable Shiga and Flexner types various bacilli have been described in different countries, which differ from the true dysentery bacilli in motility or in the property of producing gas in glucose media or by the agglutination and acid agglutination test A considerable mutation of these less stable types takes place not only *in vitro* but *in vivo* as well(7) It is quite possible that there are cases in which these unstable types are associated with the stable types (in a quarter of the cases in Cunningham and King's series) while there are others in which the former play the main role The presence of non agglutinating comma vibrios in cholera cases during certain seasons of the year adds support to our views Our knowledge with regard to dysentery in the east is still obscure In Japan(8) Komagone (A and B) types of bacilli (mannite fermenters which ferment galactose) have been incriminated in 97 8 per cent of cases whereas true Shiga infections form only 2 2 per cent of the cases

It seems to us that in between the true Shiga and Flexner types and the true *B coli* there is a gradation passing from the pseudo dysentery bacilli producing only acid in glucose and fermenting or not fermenting mannite through the paratyphoid enteritidis group which ferments glucose with gas production to late lactose fermenters The pathogenicity of these groups and their mutation both *in vitro* and *in vivo* require further study

Bacterial and Protozoal Associations

Amœbic infections were frequently associated with intestinal flagellates e g out of 200 amœbic cases the following distribution was found —

E histolytica + *Trichomonas hominis*	31
E histolytica + *Giardia intestinalis*	9

Amoebic and bacillary infections were combined together in 4 cases Among 251 cases showing intestinal flagellates the following distribution was noticed —

Trichomonas hominis	143
Giardia intestinalis	99
Giardia intestinalis and *Trichomonas* groups associated	9
Total	251

About half the samples of stools showing flagellates of the *Trichomonas* group and one fifth of those containing *Giardia intestinalis* contained both mucus and pus cells(9)

As regards secondary organisms streptococci and enterococci were present in 61 and yeast cells were found in 12 of the dysentery cases

Other causes of dysentery in Bengal

Among other causes which produce dysenteric stools may be mentioned malarial and kala azar dysentery advanced uncinarial infection heavy *Ascaris lumbricoides* infection in children ptomaine poisoning tubercular enteritis and certain forms of cholera

Balantidial and bilharzial dysentery have not been noticed in Bengal

Mode of infection

The source of infection is man (either a patient or a 'carrier') especially his stools The infection may be carried either by direct contact and carriage by food clothing or articles of daily use or indirectly by flies and water

The contamination of water supplies by surface washings during the monsoon months has been accused by some But the boiling of drinking water care of the kitchen and other precautionary measures have been taken from time to time in the Bengal jails without any great variation in the incidence of the disease

The indirect dissemination by flies has been considered to be the chief carrier of dysentery in Egypt and Macedonia where workers have observed a parallel rise in the number of flies and the increase of dysentery cases It is a fact that flies increase greatly during the summer months succeeded by the monsoon, but we have not been able to demonstrate the causal relationship here, for during the Calcutta epidemic of 1924 we dissected over 100 flies collected from the different parts of the town and cultured their intestinal contents In none of them did we get a culture of any of the incriminating organisms

Dissemination by 'carriers' must remain the most plausible method of spread of dysentery in Bengal but the monsoon increase requires elucidation The dysentery 'carrier' must be considered as a serious factor in the epidemiology of bacillary dysentery

The amoebic cases do not show the seasonal variation referred to

PROPHYLAXIS

In addition to protecting sources of water supply and food from contaminations and other measures, the stamping out of the 'carrier' condition by protective inoculation with vaccines made up of the Flexner bacilli and the intermediate group of permanent non lactose fermenters seems to be the most important measure for introduction into the jails as well as among the civil population It may be pointed out that the oral method of administering bilivaccines made from true dysentery bacilli has been found by Maitra and Basu(1926) to diminish the morbidity of dysentery in Bengal jails by 50 per cent Acute cases of bacillary dysentery in Bengal may be treated with a mixture of sera separately prepared against *B shiga B flexner* and the more pathogenic members of the Salmonella groups

REFERENCES

(1) CUNNINGHAM, J (1918) Latent dysentery *Ind Jour Med Res* Vol VI

(2) *Idem* with KING, H H (1916) Dysentery in the jails of Eastern Bengal *Ibid* Vol IV

(3) ACTON, H W and KNOWLES R (1924) On the dysenteries of India *Ind Med Gaz*, July

(4) UKIL, A C (1924) An outbreak of bacillary dysentery in Calcutta *Cal Med Jour*, July

(5) *Idem* with SEN, A K (1927) The role of certain Gram negative non lactose fermenting bacilli in the causation of clinical bacillary dysentery in Bengal *Ibid* June

(6) BYAM and ARCHIBALD'S Practice of Medicine in the Tropics Chapter 50

(7) KORTHOF G (1921) Some notes on the bacteriology of dysentery Transactions of the 4th Congress of Far Eastern Association of Tropical Medicine

(8) FUTAKI K (19 5) The dysentery bacilli in Japan and their classification Transactions of the 6th Congress of Far Eastern Association of Tropical Medicine

(9) UKIL A C (1927) The naked eye and microscopic appearance of stools containing flagellates *Cal Med Jour*, January

DISCUSSION

Lieut Col J Cunningham, I M S (British India) I would like to congratulate Dr Ukil on his very interesting paper The question of the causation of the different types of dysentery may be an elementary one, but it is none the less important for that reason Since the discovery of the different causes of this disease a discovery in which our distinguished chairman played such an eminent part opinions as to the most prevalent type of the disease have varied from time to time like the swing of a pendulum First the bacillary type claimed most attention Then the amœbic As the result of numerous investigations into the subject we, in this country, at any rate and I think also elsewhere are coming to the conclusion that the mild bacillary type is responsible for by far the greater number of the cases seen Col Forster came to this conclusion as early as 1908 and reported his results to the last big medical congress held in this country in 1909 My figures for the dysenteries found in the Bengal jails in 1914 were similar to his, namely, roughly 60 per cent bacillary and 5 per cent amœbic

More recent figures obtained by us in Madras and elsewhere have corroborated my previous results

Cols Acton and Knowles have found the same state of affairs in Calcutta and most recently a large amount of work done by the military laboratories in this country by Manifold, Little, Dunbar and others has finally incriminated the fermenting group of organisms as the most frequent cause of the disease in the army This unanimous conclusion is of the greatest practical importance

The prominence given to the amœbic type of the disease by the discovery of emetin still holds the fields in many cases Emetin, an invaluable drug when used properly has been grossly misused by the medical profession in general, with, sometimes harmful results I am of opinion, therefore, that this section, as its main duty, should emphatically record its opinion in favour of the relative infrequency of the amœbic type of the disease as compared with the bacillary and in this way do our best to place the treatment of the disease upon a better and securer foundation

Major C J H Little, R A M C (British India) Similar results have been found in Poona and Mhow, the Punjab and Bangalore, amongst British and Indian troops, Dr Ukil's diagnosis of amœbic dysentery from presence of *Entamaba histolytica* cysts is dangerous

I suggest that the Medical Research Association distribute a small cheap pamphlet such as that of Acton and Knowles' 'Dysenteries in India' General practitioners should be told of the simple method of diagnosis by taking the reaction of stools a few errors will creep in but many fewer cases would be incorrectly diagnosed and treated

Dr G Panja (Bengal) I would like to ask Dr Ukil whether his 200 cases diagnosed as amœbic dysentery had their stools examined for dysentery bacilli as well Whether the agglutination test against Flexner and Shiga organisms were tried in these cases Secondary invaders like *B fæcalis, pyocyaneus pseudo-carolinus* etc, are found and it is always best to examine the stool of a particular case repeatedly for dysentery bacilli and also to test the blood of these cases, where Gram negative non lactose *fermenters, other than dysentery bacilli, are found,* against Flexner and Shiga organisms as well as against the Gram negative non lactose fermenters themselves I hope that Dr Ukil examined the stools while they were fresh

Dr A J Noronha (Bombay) The last speaker so far as I understood him thought there existed a mild form of dysentery about which he desired to know from the President if it was deserving of special classification I have come across cases of mild dysentery from which the Flexner bacillus was isolated by me and some of which recovered under emetin which might have recovered without emetin or anything else These are exactly the cases which the private practitioner has branded as belonging to the amœbic group The Flexner type of dysentery, therefore, may in some cases prove to be extremely mild Another speaker spoke about mixed dysenteries During my six years experience in Poona I have found only one case of mixed dysentery, so that the possibility of mixed infection is a question which is not very important, one may add negligible, so far, at least as Poona is concerned *I would* like to emphasize the importance of the examination of the exudate During my conversations with Major Manifold on the subject, he suggested that I should work out the problem of dysentery prevalence *in the civil population* I have taken up

the subject very recently and my statistics would seem to point to the fact that full 90 per cent of cases of dysentery in Poona are of the bacillary type the very great majority belonging to the Flexner group The Shiga strain was isolated from cases that were usually very severe

Major P C Bannerjee (Bengal) Dr Uhil in his paper 'On the Dysenteries in Bengal' mentions only the amoebic and bacillary forms My excuse for taking your time is more for my own enlightenment as I find a lot of my learned brothers here I have seen several cases of looseness of the bowels passing blood and mucus without any pathogenic organism being detected in the stools the clinical symptoms being tenesmus, fever, griping pain all disappearing in 3 to 4 days In fact all the symptoms are those of dysentery These cases are very frequent in Calcutta Will any one of those present kindly let me know if these cases should be included in the nomenclature of dysentery or gastric influenza as Dr Russell has described as occurring amongst bacillary dysentery ?

Dr A C Ukil (Bengal) replied He admitted that he did not eliminate the possibility of a 'carrier' condition in stools showing cystic *Entamœba histolytica* When that was considered, the proportion of bacillary dysentery would increase slightly over the figure stated by him Replying to Dr Panja he said he did not culture the stools which did not show any pus cells or mucus Regarding agglutination reactions, they had already been mentioned in his paper The time of movement was noted in each case as recorded in his paper He said there was very little chance of influenzal dysentery being confused with bacillary dysentery as such cases occurred *rarely except* during widespread epidemics

He pointed out that it had been shown in his paper that bacillary dysentery in this country far outnumbered the amoebic form and that the mannite fermenting types were much more common than the classical Shiga types The importance of certain members of the so called pseudo dysentery bacilli of the salmonella groups had also been shown in his paper

Every type of case was met with in bacillary infection acute sub acute and chronic There was usually a high temperature in the first a slight temperature in the second and little or no temperature in the third form Relapses were *frequent in* dysentery

PROGRESS REPORT ON THE SPRUE INQUIRY

BY

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CONTENTS

I ABSTRACT OF RESULTS

I *M psilosis* (Ashford) is found in the majority of cases of sprue, but is present in similar proportions in cases of diarrhœa not sprue and in healthy persons

II *M psilosis* is toxic to rabbits by intravenous inoculation producing focal nephritis and death It is less toxic by other routes and generally produces localized abscesses

III The study of the hepatic function by lævulose tolerance and bromsulphalein dye tests does not bear out the contention that the function of the liver is seriously at fault

IV The study of the intestinal digestive juices indicates that the fat protein and sugar splitting enzymes are acting normally Any serious derangement of the pancreas is therefore unlikely

V The study of the morbid anatomy and histology of sprue does not reveal any pathognomonic lesions A general condition of aplasia with probable loss of absorptive power is found in the small intestine but it is difficult to say whether this is the cause or the effect of the disease

VI The blood in sprue shows changes of an aplastic type which is borne out by the condition of the bone marrow The blood picture is different from that seen in pernicious anæmia

VII Bacteriology of the intestine No bacillus peculiar to sprue has been found The commonest organism of pathogenic significance is a Morgan like bacillus A vibrio like organism has been recovered from the duodenal contents and the blood of three cases but its significance is not known Hæmolytic bacteria are commonly found in sprue stools a fact which may throw some light on the anæmia

VIII Sprue is regarded as a clinical entity quite distinct from pernicious anæmia The following reasons may be adduced —

(1) In sprue the patient is progressively and profoundly emaciated

(2) Achlorhydria though sometimes present is not invariable

(3) The blood picture differs from that of pernicious anæmia

(4) The bone marrow is generally aplastic

(5) Spinal symptoms are rarely if ever seen in sprue

(6) Recovery is frequent in sprue but rarely or never occurs in pernicious anæmia

II YEASTS AND SPRUE

Summary

(*a*) Ninety eight strains of monilia were isolated from 71 cases of sprue (mostly from one examination only)

(*b*) Twenty nine strains of *M psilosis* (Ashford) were isolated from 71 cases of sprue (40 per cent) 10 from 27 of intestinal diseases not sprue (37 per cent) 14 from 36 other diseases (38 per cent) and from about 50 per cent of the intestinal tract of healthy men and animals

(*c*) The smaller group of monilia resembling but not identical with *M psilosis* were recovered from about 33 per cent of cases of sprue and also from other diseases and in similar proportions from healthy men and animals

(*d*) *M krusei* (Cast) was present in about 50 per cent of sprue and the other human diseases and in a smaller proportion of healthy men and animals

(*e*) The distribution of these classes of yeasts was similar in sprue to that in other diseases and there was no undue frequency of any type of yeast in any diseased or healthy condition and therefore there is no evidence to show that any of these monilia bear a causative relation to sprue

(*f*) We are however prepared to believe that the fermentative conditions set up by yeasts in the intestinal canal of sprue patients may play a part in producing the symptom complex of the disease

(*g*) Our study of the structure and life history of intestinal yeasts leads us to the view that they have been over-differentiated and that the human intestinal yeasts are relatively few in species and easily classified on broad lines into a few distinct types

(*h*) Knowing the variability of strains by subculture, it is safer to accept this as the explanation rather than create a large number of species dependent on characters which are known to be inconstant

Conclusion

M psilosis (Ashford) has been found in Bombay—

(*a*) To be present in 10 per cent of cases of sprue

(*b*) To be present in similar frequency in intestinal diseases (not sprue) other miscellaneous diseases and in healthy men and animals

(*c*) There is no evidence to show that it or any other of the yeasts studied has any causative relation to sprue

III Animal Experiments with Yeasts

When *M psilosis* (Ashford) is injected into the peritoneal cavity of guinea pigs on a single occasion, it gives rise first of all to injection and a little serous exudation Later on lymph is thrown out and plastic peritonitis results This is at its height about the third day and then begins to subside During this early period yeasts may *frequently be recovered from the heart blood The animals almost invariably* survive a single injection and, if killed at the end of a week or ten days the peritoneal reaction has passed and yeasts cannot be recovered by culture In a few cases encapsuled foci containing degenerate yeasts are found The blood and viscera are not found to be infected after the first few days following injection but the process remains strictly localized No toxic symptoms are noticed When repeated intraperitoneal injections are made at intervals of a few days a condition of severe peritonitis is set up and the animals die from this cause On post mortem examination the abdomen contains much plastic exudate sometimes with pus formation and the exudate is often invaded by coliform organisms together with the yeasts

A generalized monilia septicæmia with deposits in the viscera sometimes results but more generally the process is localized

When *M psilosis* was injected into the substance of the tongue of a rabbit (one case) no ill effects were noticed locally and there was no infection of the blood or viscera

Whether exaltation of virulence takes place by passage

A series of seven guinea pigs was inoculated from one to the other to see if exaltation in virulence took place by passage The inoculations were done by the peritoneal route and the animals killed after three days The yeast was found locally and in the heart blood in the majority of cases, but the virulence of the monilia was not found to be exalted

Six monkeys were devitalized by being fed on a diet deficient in vitamin C for several weeks until symptoms of incipient scurvy appeared They were then infected as follows —

Monkey I Fed on fæces of four sprue patients
II Do Do
III Do Do
IV Do Do
V Fed on *Monilia ashfordi* culture Bombay Type VIII and *Monilia ashfordi* culture Bombay Type CCLVIII
VI Fed on *Monilia ashfordi* culture Bombay Type VIII and *Monilia ashfordi* culture Bombay Type CCLVIII

The results were as follows —

I Died of dysentery from which organisms resembling *B faecalis alkaligenes* and *B morgani* were isolated It showed no signs of sprue

II It suffered from a mild attack of dysentery but recovered from the same It is still alive and shows no signs of sprue

III This monkey did not suffer from dysentery nor has it developed signs of sprue It is still alive

IV It died with symptoms of dysentery no dysentery organism was isolated from its stool It showed no signs of sprue

V It suffered from dysentery and had become extremely emaciated and ill It was therefore killed No dysentery organisms were isolated No evidence of sprue

VI It died of dysentery—but no dysentery bacilli were isolated Did not develop sprue

Some strains of *M psilosis* when inoculated intravenously into rabbits proved to be profoundly toxic and killed the animals in a few days At the post mortem the blood and principal viscera were found to contain numerous yeasts in a state of active growth The brunt of the attack falls on the kidneys which are found to be studded with large numbers of minute foci in which yeasts are multiplying The rabbits develop convulsions and coma before death If a very small dose is given and the animal recovers it is found that sclerosed areas due to focal destruction of the kidney cortex are left behind Monkeys rabbits guinea pigs and white mice fed on these toxic strains of yeasts do not suffer any ill effects

In none of the animals however inoculated and whether in a condition of vitamin C deficiency or otherwise was there any development of an intestinal condition resembling sprue nor was any condition of anæmia produced in these animals which were specially examined for this condition

The conclusion is that though *M psilosis* is undoubtedly toxic for some animals especially by intravenous infection a sprue like condition was never set up as a result of any of our experiments

IV THE BACTERIOLOGY OF THE ALIMENTARY TRACT IN SPRUE

(a) *Duodenal (6) and gastric (1) contents during life, removed by duodenal tube*

From these seven cases, the following strains of bacteria have been studied —

1	Streptothrix	2 strains
2	Cocci	16 „
3	Gram negative bacilli, coliform	14 „
	„ „ non coliform	2 „
4	Gram positive bacilli—	
	Ærobic spore bearer	1 „
	Ærobic non sporing bacilli	14 „
5	Anærobic bacteria	*Nil*

The coccal types were—

Staphylococci, 9 strains	All Gram positive
Diplococci, 7 „	Gram positive 2
	Gram negative 5

Four of the staphylococcal strains were hæmolytic Three of the diplococcal strains were hæmolytic

The Gram negative coliform bacilli were either *B coli* or one of its near congeners None of them were hæmolytic

The Gram positive bacilli have not been studied sufficiently to determine their species and only one of them was hæmolytic

(b) *Fæcal flora*

The bacterial content of about 70 cases of sprue was studied

The organisms were divided into—

(a) Cocci

(b) *B coli* and its cogeners (lactose fermenters)

(c) Coliform bacilli (lactose non fermenters)

(1) Group *Eberthella* (acid in glucose)

(2) Group *Salmonella* (acid and gas in glucose)

The former group included *B fæcalides, aseptcus, belfastiensis* and *meta dysentericus*

No organisms identical with recognized pathogenic species were isolated Most of the group were indol producers

The *Salmonella* group included a large number of strains, but with the exception of *B morgan* none were recognized pathogenic types

B morgan was found fairly frequently, but was irregular in its reactions and serological relations

Anærobic bacteria — The investigation of these has only been begun recently and the following results are noted —

No anærobes were found in any of the seven samples of duodenal contents

Practically all samples of stool contained anærobes of *B welchii* type and all were strongly hæmolytic

Hæmolysins —The fresh fæces of 12 cases of sprue and sprue like anæmias were examined for free hæmolysins by the dilution method

Six of them were hæmolytic in one case up to a dilution of 1 in 100 000 in others to a much less degree

All 12 fæces whether containing free hæmolysins or not were found on culture to contain hæmolytic bacteria

In one case the fæces from different levels of the alimentary canal after death were examined and abundant hæmolysis acting in a dilution of 1 in 100 000 was found in the stomach duodenum jejunum ileum and colon Eight strains of hæmolytic bacteria were found in 34 ærobic strains from the duodenal contents and 25 out of 77 ærobic strains isolated from the fæces The hæmolytic power of these were not measured and in many cases it was quickly lost on subculture The hæmolytic organisms were in some cases cocci and in others Gram negative or Gram positive bacteria whilst the anærobes isolated were nearly always hæmolytic

Remarks This aspect of the problem was undertaken in the hope that some organism would be found regularly or frequently associated with sprue and its influence in the production of the characteristic alimentary symptoms determined

In this hope we have been disappointed but the investigation is still incomplete and much more requires to be done

During the last year we have paid more particular attention to the hæmolytic organisms in the hope that some light might be thrown on the production of anæmia which is so marked a symptom of sprue We have borne in mind the results obtained on these lines in pernicious anæmia and our results bear out the general trend of opinion regarding this disease Free hæmolysin and hæmolytic bacteria are present in a considerable proportion of sprue cases and it is possible that this factor may have some influence in bringing about the aplastic condition of the marrow

A vibrio like organism of unrecognized species was isolated from the duodenal content of one case and from the blood of two others all during life and the nature of this organism is being investigated Except for these two cases the blood has always been found free from bacteria and no spirochætes have been found by dark ground illumination or by staining methods

There is no evidence so far that any particular micro organism is causally related to sprue, but this by no means vitiates the hypothesis that sprue is the result of an alimentary infection

V THE BLOOD IN SPRUE

The total number of sprue cases examined during the course of this year were twenty eight

The hæmoglobin percentage was worked out on Sahli s principle

Blood changes do not manifest themselves at the commencement of the disease Early cases show slight anisocytosis with a slight decrease in the number of red blood cells and a slight fall in the hæmoglobin percentage

Blood picture

Most of the advanced cases present a constant blood picture There is marked anisocytosis —the megalocytes preponderate along with a few microcytes The poikilocytes are few and polychromatophilia is present but generally scarce A noteworthy aspect of the blood picture is the total absence of nucleated red cells a feature which distinguishes the sprue anæmia from pernicious anæmia

Red blood cells —Between			
	1	and 1 5 millions	5 cases
	1 5	2	2
	2	2 5	1 case
	2 5	3	3 cases
	3	3 5	6
	3 5	4	*Nil*
	4	4 5	7 cases
	4 5	5	1 case
	Over 5	millions	3 cases

In one case the red blood cells were only 400 000 The patient was on the verge of death and had suffered from a blood crisis

Average R B C count of 28 cases = 3 247 395 per c c

Hæmoglobin —Between			
	30	and 40 per cent	4 cases
	40	50	3
	50	60	3
	60	70	4
	70	80	7
	80	90	4
	Over 90 per cent		3

In one case the hæmoglobin recorded was as low as 10 per cent

Colour index —The colour index is generally over 1 but this is not constant in many cases the colour index falling below 1

0 7	4 cases
0 8	3
0 9	6
Between 1 and 1 1	12
1 2	1 case
1 3	1
1 8	1

White blood cells —The white blood cells show a diminution in the total count

Between 2 5 and 3 thousand		1 case
„ 3 „ 3 5 „		2 cases
„ 3 5 „ 4 „		2 „
„ 4 „ 4 5 „		4 „
„ 4 5 „ 5 „		*Nil*
„ 5 „ 5 5 „		4 cases
„ 5 5 „ 6 „		3
„ 6 „ 6 5 „		3 „
6 5 7 „		*Nil*
7 „ 7 5 „		*Nil*
7 5 „ 8		2 cases
„ 8 and 8 5		1 case
Over 10 000		5 cases

Average of 27 cases = 6,828 per c c

Highest number of W B Cs 20 312 per c c

Differential count —The differential count generally shows a relative increase in the percentage of lymphocytes

Taking an average the polymorphonuclears are 58 5 per cent and the lymphocytes 40 4 per cent The other white blood cells fall within the normal range and especially the eosinophiles are conspicuous by their being within the normal limits

Conclusions

In a former progress report on sprue we gave the figures for 25 consecutive cases The averages for these were as follows —

R B C	3 243 490 per c c
Hb	65 1 per cent
Colour index	1 0

The average leucocyte count was 6 367 per c c and the average differential count was—

Polymorphonuclears	49 7 per cent
Lymphocytes	42 5 „
Large mononuclears	4 9 „
Transitionals	1 7 „
Eosinophiles	1 2 „

The correspondence between the two series 25 before and 27 now is very close and we are in a position to draw certain conclusions on these figures Profound anæmia where the red cells are below a million is rare in sprue but is met with in the terminal stages sometimes as a result of a blood crisis where the count may fall by two million per c c within a week Severe anæmia counts between 1 to 2 million,

ON THE THERAPEUTIC VALUE OF BLOOD TRANSFUSION IN SPRUE ANÆMIA

BY

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ONE of the most mysterious distressing and frequently fatal features of sprue is a *rapidly developing anæmia of the Addisonian type*

In a certain proportion of sprue cases, especially in those of long standing and in patients over fifty years of age, this pernicious anæmia (for such it is) may be the outstanding feature of the disease Usually the anæmia is secondary to the diarrhœa and emaciation, developing gradually and progressing slowly to an extreme and fatal degree but there are other cases, familiar to the tropical practitioner, in whom a sudden liberation of hæmolytic toxin takes place with the production of a *rapidly progressive anæmia which may prove fatal in a few days In the writer's* experience of fatal cases of sprue occurring in the Hospital for Tropical Diseases during the last seven years, only one case died of inanition, two of perforation and general peritonitis while five died of this pernicious anæmia

Though there can be little doubt to the practised eye that the accompanying anæmia in sprue and Addisonian anæmia are two distinct entities yet there are at present no outstanding features by which the one can be distinguished from the other on any *reliable grounds It is probable that the physical characteristics of the* anæmia in both cases are identical The colour index in both diseases is above 1, the reduction of red blood corpuscles may be extreme the morphology and degeneration of the red cells are identical, and even megaloblasts, which distinguish the Addisonian anæmia, are occasionally to be found in sprue The Van den Bergh reaction provides no means of differentiation and, in both, a relative *leucopenia* accompanies the extreme anæmia

Addisonian anæmia generally runs its course with intermissions unchecked and cannot be permanently influenced by dietetic measures, as far as is known It is otherwise with sprue in which blood regeneration sometimes occurs completely and entirely on no other grounds than dietetic restrictions

In our opinion no other therapeutic measure in sprue has given such brilliant results as has blood transfusion

The indications for this measure are self evident on the analogy of pernicious anæmia, but we would emphasize that, whereas in the latter the results are merely temporary, in sprue they appear to be permanent It is true that in some cases it has been necessary to repeat the transfusion as many as three times but we would stress the ultimate and apparently permanent effects that accrued even in cases which appeared to be *in extremis*

In the last five cases of sprue anæmia treated by this measure, surprising and lasting results have been obtained in every one

It is necessary for the sake of clarity that the protocols of these five cases should be given somewhat in detail—

Protocols of Cases

I This is of a gentleman of 61 years of age who had lived in the Straits Settlements for 32 years He had been suffering from sprue for nine years and was invalided home with this complaint in 1919 being then extremely ill From then onwards till admission to hospital on 24th March 1926 he had suffered off and on from acute relapses of sprue with sore tongue and frothy stools At the commencement of 1926 severe anæmia set in and he was frequently attacked by dizziness and faintness For several weeks his mentality had been completely deranged and finally he became semi conscious with incontinence of fæces On admission to hospital he was comatose and did not regain consciousness for 14 days He appeared to be *in extremis*, his skin was tinted a lemon yellow colour and he was extremely emaciated The blood count at this time was as follows —

Red blood corpuscles 1 100 000 hæmoglobin 30 per cent white cells 2,000 an extreme degree of poikilocytosis was present in blood films, while normoblasts were comparatively numerous

Two transfusions of citrated blood were given on 29th March 550 c cs and again on 9th April, 400 c cs After the second, improvement became daily more obvious, consciousness was partly regained on 11th April but convalescence was checked by an attack of right basal lobar pneumonia on 7th May, which lasted one week and from which he made a rapid and successful recovery There was a short relapse of sprue symptoms with diarrhœa and meteorism in the middle of June, but in the first week of July the blood was fully restored to normal, the red blood cells numbering 5,100 000 and hæmoglobin 100 per cent The blood pressure had risen from under 100 mm to 160 mm systolic pressure

After leaving hospital on 22nd July 1926 he has been under observation and no return of sprue symptoms has been observable He is now (October 1927) of good colour possesses considerable physical vigour, can walk and take an active part in social affairs His blood remains normal and his weight has increased from 8 st 4 lbs in May 1926 to 11 st 4 lbs, a total increase of 42 lbs

II A gentleman of 72 years of age who has lived over 50 years tea planting in India returned to England in April 1926 For one year previous to retirement he had suffered from sprue symptoms and had lost 42 lbs in weight

Early in 1927 severe and progressive anæmia was noted and when seen on 8th April he presented [illegible]

[illegible]

[illegible]

Admitted to hospital on 21st April 1927, and, being in a critical condition, he was transfused with

500 c cs of group IV citrated blood the next day There was a slight reaction of temperature, but no marked improvement in the patient's mental condition or in the blood count resulted A second transfusion was given on 6th May with 120 c cs of citrated blood Thereafter with few intermissions, such as recurrent aphthæ on the tongue and attacks of diarrhœa he continued to improve gradually On 30th June, the red cells numbered 4,000,000 and the hæmoglobin rose to 75 per cent The diet was then greatly increased Shortly before discharge, the hæmoglobin percentage was 80 and the red cells 4,680,000 The improvement has since been maintained there has been no return of sprue symptoms In August 1927 he was vigorous and well, red cells numbered 5,290,000, hæmoglobin 100 per cent His weight is now 11 st 11 lbs

III This is probably the most remarkable case of the series A gentleman of 54 years of age had lived for 25 years in the Philippines and in Hongkong and is known to have suffered from sprue off and on more or less for 20 years Apparently he had had a great deal of diarrhœa and had not passed a normal motion for years Towards the close of 1926 the anæmia became more and more apparent and he had to leave Hongkong in January 1927 On board ship his condition greatly deteriorated so that on 5th February he was landed almost *in extremis* Semi conscious with evident air hunger, he presented the most extreme degree of anæmia it is possible to witness The hæmoglobin was estimated at 10 per cent, red blood corpuscles 400,000, white cells 3 280 Degenerative changes in the red cells were present, but no nucleated reds On February 7th, after failure to procure enough serum for blood grouping, 350 c cs of citrated blood (group IV) were transfused The response was immediate and remarkable On careful dieting the sprue diarrhœa ceased, and return of physical strength and mental vigour became day by day more apparent The blood pressure which was 80 mm systolic rose rapidly till by the end of March it was 124 mm Within a week of the transfusion the hæmoglobin was 35 per cent, red blood corpuscles 1,800 000, white cells 6 200 and numerous normoblasts and megaloblasts were seen The patient exhibited continuous improvement, marred only by occasional attacks of gout in his hands and feet which became evident, curiously enough, almost immediately after the transfusion On discharge from hospital on 1st April 1927, the hæmoglobin was 100 per cent, red blood corpuscles 4 450 000 and weight 10 st 3 lbs Since that time the improvement has been maintained He is now, October 1927, in good condition weighing 12 st 4 lbs, has no visible sprue symptoms and the blood count remains practically normal

IV A lady of 62 years of age resident in Shanghai for 28 years, was admitted to hospital first on 27th May, 1926 She had suffered intermittently from sprue for 15 years and had been becoming progressively weaker, more emaciated and anæmic Loss of weight was over 3 st The weight was 7 st 2 lbs and in addition to other sprue symptoms, she exhibited a most curious diffuse pigmentation on the forehead cheeks hands and abdomen The blood count was then red blood corpuscles 1,000 000, white cells 4 000 and hæmoglobin 40 per cent, the usual morphological changes being present, but no normoblasts

On this occasion she improved temporarily on dietetic measures and iron and arsenic injections and was discharged with a hæmoglobin content of 75 per cent and 3 500,000 red blood corpuscles The anæmia, however, returned in an acute form and on 30th June, 1927, she sought readmission to hospital presenting the typical picture of pernicious anæmia with lemon tinted skin and œdema of the face and ankles The anæmia was fairly extreme, red blood corpuscles 2,000,000, hæmoglobin 50 per cent

Being of group IV she was transfused on 5th July, 1927, with 380 c cs citrated blood. The response was almost immediate so that in October she presents an entirely altered appearance, the hæmoglobin being 80 per cent and the red cells 4,340,000 The change in mentality and vigour has been as striking as the improvement in the blood condition There has been no diarrhœa, though the tongue and mouth have been irritable from time to time

V This is a gentleman of 45 years of age who had resided 22 years in Hongkong During the last 1½ years he had suffered greatly from acute sprue symptoms and had lost over 28 lbs in weight Invalided from Hongkong in April 1927, he landed in England in an extremely poor state He was admitted to hospital on 7th June, 1927, for blood transfusion His hæmoglobin percentage was then 70 and the red blood corpuscles 2,600 000, the usual degenerative changes were present and scanty normoblasts were seen On June 15th blood transfusion was performed, but on account of its small

calibre the vein had to be cut down upon and exposed so that only 70 c cs of citrated blood could be successfully introduced This small amount however appeared to be quite sufficient to stimulate blood regeneration After 14 days in hospital he retired to convalesce in the country and when seen again in August 1927 he gave the impression of vigorous health He had increased over 1 st in weight since leaving the hospital had no ascertainable sprue symptoms a hæmoglobin percentage of 100 and 5 1[illegible]0 000 red cells

The deductions which may be made from a study of these cases appear to be the remarkable and lasting effects of blood transfusion It is apparently not due so much to the mechanical replacement of destroyed blood corpuscles as to stimulation of the hæmapoietic system It will be noted that in two instances more than one transfusion may be necessary in order to obtain the desired result The impression is certainly obtained that the actual amount of blood injected is a matter of secondary consideration In Case V cited brilliant results appear to have followed the injection of a comparatively small quantity namely 70 c cs We are of the opinion that in very severe cases of sprue anæmia with an extremely low blood count such as Case III the injection of a larger amount of blood than 300 c cs should not be attempted

The stimulating effects of blood transfusion may be seen in Case I who successfully surmounted an attack of lobar pneumonia during convalescence and Case III who developed acute gout subsequent to injection

A comparatively short period has elapsed since the final case was observed so that one cannot state whether relapses of actual sprue symptoms are liable to recur but available evidence would seem to point to the fact that not only is the anæmia permanently cured but also evident symptoms of acute sprue are banished by blood transfusion It is hardly necessary to observe that in order to obtain the full benefits of blood transfusion the strictest dietetic measures are necessary as in ordinary sprue The regeneration of the blood is greatly aided in our opinion by the exhibition of *Liquor arsenicalis* (Fowler s solution) which has been given to all the cases cited The initial dose should be 1 minim daily and it is gradually increased till the patient is taking 15 minims daily The arsenical treatment must be continued for two weeks and resumed after the pause of a fortnight No symptoms of arsenical intoxication have been seen in these cases but it is a possibility which must be carefully guarded against Occasionally as in Case II it has been found advisable to supplement the action of *Liquor arsenicalis* by intravenous injection of novarsenobillon 0 1 gramme at weekly intervals

In view of the claims of the beneficial effects of liver diet in Addisonian anæmia now being made by Minot and Murphy in America it may be stated that the value of liver soup has long been recognized in sprue All these patients have received as an essential part of their dietary 8 ounces of strong liver soup daily

In every case a reaction was noted immediately following blood transfusion A rise of temperature from 100°F to 102°F occurred in one instance (Case IV) with a rigor and it may be stated in general terms that the more marked the reaction, the more immediate the results

An icteric tint of the skin and sclerotics was observed the day following the transfusion, in Case IV a serum rash with urticaria broke out on the third day subsequent to transfusion and lasted three days

No other serious reactions were noted

Technique Employed

The simplest technique has given the best results in our hands citrated blood only has been used For this purpose we use two Florence flasks of 500 c cs capacity with a mark at the level of 330 c cs Two needles of uniform bore with short bevel a tube funnel with suitable rubber connections and needle for giving blood to the recipient are necessary A tourniquet such as in a 'Tycos' blood pressure apparatus is used for constricting the arm One hundred and sixty c cs of sterile citrate solution (3 8 per cent in normal saline) must be provided

Into each Florence flask 80 c cs of citrate solution should be placed The tourniquet should be applied to the donor's arm and pressure exerted up to 80 mm of mercury The veins having been made prominent in this manner, a broad bore needle (size No 10, Maw) is inserted into the vein and the blood as it spurts forth is collected into the flask containing the citrate, which should be gently rotated so as to ensure the proper mixing of the blood If more than 250 c cs of blood are required the second Florence flask should be substituted when the blood has reached the 330 mark When sufficient has been collected, the tourniquet pressure should be released and the needle withdrawn The flasks containing the blood should be kept in basins of hot water at 105°F a temperature which will not injure the blood but will help to counterbalance the heat loss during the subsequent transfusion

The armlet is now applied to the recipient and pressure exerted up to 60 mm of mercury After preparation of the skin, hot sterile saline should percolate through the funnel and tube so as to warm them thoroughly and a small quantity of the citrated blood poured in After expelling any air bubbles present in the tube an intravenous needle should be inserted into the recipient's vein, directly blood begins to flow showing that the vein has been correctly entered, its shaft should be attached to the rubber tubing of the funnel and the blood permitted to flow slowly into the recipient's vein In order to permit of this, the pressure of the [illegible] be released At least twenty minutes should be occupied in running [illegible] ng exercised to keep it at the correct tem [illegible] to flow properly may lie in the collapsed and contracted *condition of the veins of the recipient* *By making* the armlet tight enough to arrest completely the circulation for about ten minutes the collected carbon dioxide will cause a local vasomotor relaxation, on the pressure being reduced to that of the diastolic pressure of the recipient the maximum dilatation of the veins will occur It is upon the attention to detail in dilating the recipient's *veins that the* success of the injection depends, and it is for this reason that the armlet of a blood pressure apparatus is preferable to a tourniquet

4 F

The grouping of these cases has been carefully performed previous to transfusion, save in Case III, when the extreme urgency did not permit of this being done In Case IV an anomalous result of auto agglutination of the patient's red blood cells was noted This was sufficiently alarming to cause a postponement of the transfusion It has been thought advisable to append a note on the investigations into this phenomenon by P H Martin

This paper has been a considerable time in preparation and, since it was written a communication by Carmichael Low and Cooke(1) has appeared which confirms nearly all that has been said here

Our best thanks are due to Dr A L Gregg for his help in blood transfusions and assistance in the technique of the operation.

REFERENCE

(1) Low, G C, and Cooke, W L (1927) *Lancet*, II, pp 960 961

NOTE ON THE AGGLUTININ SYSTEMS

BY

P H MARTIN.

One of the above recorded cases, Case IV, presented signs of auto agglutination The system was unfortunately not at all thoroughly worked out, but its dependence on low temperature was demonstrated, though not its reversibility nor was its maximum titre tested As far as was ascertained, the system agreed with reports of similar cases as worked out by Warrington Yorke in Trypanosomiasis'(1), and as recorded by other workers as Clough and Richter(2), and Cohen and Jones(3)

On a previous admission in December 1926, Case IV was found to belong to group IV (Moss) No transfusion was, however made at that date, nor prior to 5th July, 1927 On 4th July a sample of the patient's blood was taken, so that the routine test of the recipient's serum with donor's corpuscles might be made, and a group IV donor sent for Agglutination was found to occur, and the transfusion was postponed

On the next day a further sample of blood was taken Auto agglutination, at and below room temperature, was found to occur and the patient's serum similarly agglutinated the cells of another group IV person By chance some of the previous day's serum was still available This serum was found to have lost its power to agglutinate either the patient's own washed cells or other group IV cells This serum had (1) been in contact with the patient's cells in the coagulation tube for several hours (this may have enabled all the agglutinins to have been absorbed from the serum), and (2) had been kept at low temperature during the night This may have destroyed some part of the system The idea of testing which of these factors was operative did not occur at the time.

The patient's cells were not agglutinated by the sera used of groups II III and IV This agrees with the findings of Clough and Richter(2) and Cohen and Jones(3), who found that the cells of their patients behaved normally

No group I blood was available, and no attempt was able to be made to look for a third iso agglutinin 'C' of Guthrie and Huck(4) and Simson(5) 'X' of Coca and Klein(6) Fresh supplies of bloods of groups II, III and IV were available and have also been used in the subsequent investigations

In a previous case showing auto agglutination, in the cold, for whom blood transfusion was very desirable, Mr Geoffrey Keynes kindly advised that the blood should be given, especial care being taken to introduce the blood slowly and to stop the flow should any signs of incompatibility be seen This transfusion had been carried out with complete and uneventful success

The transfusion of Case IV was therefore carried out, and only a mild reaction of incompatibility followed Hæmoglobinuria lasted under 24 hours, and was not severe as her blood counts show—

	July 4th	5th	6th	18th
R B C	2 000 000	Transfusion	2 460 000	3 410 000
Hæmoglobin	60 per cent	with 380 c cs citrated blood	60 per cent	70 per cent

It is hoped that it was only a failure to keep the ingoing blood quite up to blood heat that allowed any hæmolysis to occur

On October 17th Case IV's blood picture was—

R B C	4 340 000
Hæmoglobin	80 per cent
W B C	4 400

Van den Bergh Positive indirect, a little under 1 unit During the next few days her blood was examined and compared with those of normal persons and owing to the kindness of Dr G Carmichael Low, with the bloods of two cases the first a case of sprue with anæmia, in a middle aged man who had reacted to a trans fusion in August with a considerable hæmoglobinuria and later great improvement the second a case of Addison's anæmia One sample of blood from Case III was also used for agglutination tests only

TECHNIQUE

Blood groups were determined by the method of Dyke(7) except that a hanging drop was only used where evaporation was rapid

Tests for hæmolysis were made by the method of Troisier(8) which consists in mixing one drop of red blood corpuscles with twenty drops of serum and incubating the mixture for half an hour at 37°C The mixture was centrifuged and hæmolysis looked for

Tests for the presence of a hæmolytic amboceptor were made by the method of Widal and Weissenbach(9) which method examines for the presence of adsorption of amboceptor by the red cells during the above test The cells are washed free from serum normal saline is added and complement (guinea pig serum) incubation for a second half hour at 37°C follows centrifugalization and

exam nat on for hæmolys s Th s method includes very thorough controls of the 0·9 per cent saline of the gu nea pig serum and of normal ser m both w th and w thout guinea p g serum

To obta n washed cells bloo l from the warmed syr nge used for veni puncture was injected into warm (37 C—40 C) c trat sal ne Three samples of the sera were prepared by allowing the blood to clot at 37 C at room temperature and in the ice chest

The m xture of cells and serum was observed for agglutinat on at (1) room temperature (°) after one hour n the ce chest (n cap llary tubes) and (3) after one hour in the ice chest and a subsequent hour at 37 C (also in cap llary tubes)

No trace of auto agglutination was seen in any of the pathological sera nor in the controls No group IV cells were agglutinated and the group II patho logical serum did not agglutinate normal cells of groups II and IV

No hæmolysin nor hæmolytic amboceptor which could act on group IV cells was found

Where mixtures of cells and sera which would be normally incompatible owing to their iso agglutinins were observe l for hæmolysis and for hæmolytic amboceptor the results were positive usually in the case of the pathological sera and sometimes with the normal sera This agrees with the findings of Jones in 1921(10) but our present results have been too irregular to justify any deductions from them

Where incomplete hæmolysis occurred after a mixture had been at 37°C for over an hour the sera after centrifugalization were still found to show the reactions normal to their iso agglutinin content

The complete disappearance or latency of the auto agglutinin system des cribed above is in accord with the experience of Dr G W Goodhart In January 1927 at University College Hospital he observed in a case of Addisonian anæmia the presence and later with improvement of the blood picture the disappearance of auto agglutination We are indebted to Dr Goodhart for this information which he gave in conversation about his case and for access to his unpublished notes Warrington Yorke(1) records the observation of Dutton and Todd of the simultaneous disappearance of trypanosomes and auto agglutination from the blood of an European Clough and Richter(2) suggest that in their cases it is probably not a pathological phenomenon but an individual hereditary peculiarity

It has been thought wise to record the observation of auto agglutination occur ring in sprue and also the possibility of transfusing blood into such a case the very greatest care being taken to maintain the temperature of the entering blood at that of the body

The intermission of the phenomenon during a remission or possible cure ' of the anæmia is noted

The presence of any hæmolytic system which can act on cells of group IV (Moss) (on which the auto agglutinin can act) has so far eluded detection during a remission but was not looked for during the time of relapse when auto agglutination was seen

It is hoped that another opportunity of studying the phenomenon of auto agglutination will occur

REFERENCES

(1) Yorke W (1911) *Ann Trop Med Hyg and Parasit* Vol IV p 5?9

(2) Clough M C and Richter J M (1918) *Johns Hopkins Hospital Bulletin* Vol XXIX p 86

(3) Cohen H and Jones I (19?4) *Lancet* 2 p 853

(4) Guthrie C G and Huck J G (1923) *Johns Hopkins Hospital Bulletin* Vol XXXIV, pp 3 80 128

(5) Simson F W (19?6) *Jour Path and Bact* Vol XXIX pp 3 ?7?

(6) Coca A F and Klein H (19?3) *Jour Immunol* Vol VIII p 47?

(7) Dyke S C (19?2) *Lancet* Vol I p 579
Idem (19?7) *Ibid* Vol II p 910

(8) Troisier J (1910) *These de Paris* No 429

(9) Widal F and Weissenbach R J (1913) *C R de la Soc de Biol* Vol LXXV p 18

(10) Jones B (1911) *Amer Jour Dis Child* pp ?? 598

PANCREATIC FUNCTION IN SPRUE

BY

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Originally the pale colour and the bulkiness of sprue stools were attributed to defective biliary secretion leading to defective assimilation of fat Actual estimation of bile pigments in the fæces showed that they were present in normal amounts Later the excess of fat in sprue stools was considered to be due to defective pancreatic secretion Work of various workers on this point has yielded conflicting results and in our opinion this conflict is due to the methods employed in estimating the pancreatic secretion For example Pratt and Spooner used Schmidt Einhorn thymus test and the Sahli glutoid salol capsules while Brown measured the diastatic activity of urine and fæces Since the modern duodenal tube renders possible a direct and more reliable examination of pancreatic ferments poured into the duodenum we undertook to re investigate this point We also undertook to analyse the fat content of the fæces to see if it would yield any definite information on the subject of pancreatic efficiency Employing Saxon s wet method of fat analysis of fæces we found that in only one of our series of seventeen cases of typical clinical sprue did neutral fat exceed 60 per cent of the total fat content while all cases except one showed normal splitting of fat Quantitative estimation of diastase trypsin and lipase of duodenal contents of five cases that we examined showed these ferments to be present in normal amounts We have therefore come to the conclusion that the pancreas as far as its external secretion is concerned functions normally in sprue

We also would like to mention that the total fat content of 14 out of our series of 17 sprue cases was high ranging from 37 8 per cent to 60 7 per cent of the total dry matter All our cases were on milk diet and as it is the mostly used diet in the condition it therefore occurred to us that the high fat content of the fæces might have something to do with the milk consumption considering fat constitutes more than 50 per cent of solids of milk other than sugar We therefore examined the fæces of 17 led cases on milk and suffering from diseases other than sprue, i.e ,

aneurysm of aorta, hemiplegia, rheumatic arthritis, etc We found that the fat content of these cases ranged from 29 7 to 73 6 per cent Neutral fat and split fat ratios agreed with similar ratios of sprue stools As a result of these findings we think that high fat content of fæces in sprue does not disclose anything that is peculiar to sprue

LIVER FUNCTION IN SPRUE

BY

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AND

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THERE are scattered references in the literature to the effect that the liver is affected in sprue Brown found the liver to be reduced in size Begg considered reduction in the size of the liver to be a cardinal sign He did not find the liver to be cirrhotic, but normal and merely reduced in size More recently Mikeladze also reported reduction in size of the liver Wood summarizing the literature remarks ' the liver is vaguely described as atrophied, but there seems to be little evidence *that this atrophy is anything more than that shared by all the tissues* '

We, therefore undertook an investigation to see if modern functional tests would throw any light on the efficiency of the liver in sprue

Thirteen cases that could be definitely described as sprue were studied The functional tests employed were—

1 Lævulose tolerance tests, based on the relation of the liver to carbohydrate metabolism

2 The Van den Bergh reaction depending on the secretion of bile by the liver

3 Nitrogen partition of the blood based on the relation of the liver to protein metabolism

4 Bromsulphalein dye test of Rosenthal and White based on the specific action of the liver in extracting this dye from circulation in the blood

Nitrogen partition did not reveal any inefficiency The bromsulphalein dye test yielded negative results, except in one case in which it may well have been due to the extreme lowering of vitality on account of approaching dissolution, the test in this case was done three days before the patient died We found an increase in serum bilirubin in six of our 13 cases, as shown by the indirect Van den Bergh reaction This increase of bilirubin, in our opinion, was not due to faulty action of the liver, but to increased destruction of erythrocytes as borne out by cell counts If the liver had been at fault, the dye test would have shown a parallel retention Seven of our cases gave abnormal lævulose tolerance curves But the work of Mann and Bollman has shown that the lævulose tolerance test is not specific for the liver

They have shown that when the glucose or lævulose test is done on an animal which has been fasted for four or five days, the rate at which the blood sugar level returns to normal is very much retarded much more so than in an animal four fifths of whose liver has been removed, in the latter case retardation is only very slight, not lasting more than 60 minutes In sprue, inanition may more likely be responsible for the abnormal lævulose tolerance curves than the condition of the liver So if we ignore the results of our lævulose tolerance test, on account of the non specific nature of the test we find that the other three tests give parallel results showing *that in sprue the liver is not affected to such an extent as to show* impairment by liver function tests

DISCUSSION

Dr J P Bose (Bengal) I have only a few words to say regarding the sugar tolerance of a few cases of sprue treated at the Carmichael Hospital for tropical diseases in Calcutta *I tested a series of 10 cases of varying degrees of severity* The average initial fasting blood sugar level was found to be 0 14 per cent which is much over the normal level The blood sugar began to rise after a test meal of 50 grammes of glucose in half an hour's time it went up to an average of 0 156 per cent in 1 hour's time to 0 165 per cent, in one and a half hours to 0 170 per cent, in 2 hours it slowly came down to 0 160 per cent and in 3 hours time it came down to 0 150 per cent only There was no glycosuria three hours after the glucose meal was taken The results drawn on graph paper represented a long drawn out, flat top blood sugar curve, indicating definitely a defect in the sugar storage mechanism and sugar utilisation by muscles and tissues I am investigating these cases at the suggestion of Col Megaw, but we are not yet in a *position to say yet whether this defect in carbohydrate metabolism is* primary or secondary All these patients were Europeans and Anglo Indians

Dr R B Tandan (Jodhpur State, India) I want to describe some very specific and sure modes of treatment in the northern part of Rajputana carried on by the country physicians

Loha parpati is made thus —Take one part of metallic mercury obtained from Shingraf (red sulphide of mercury) take 2 parts of purified sulphur (amlasar gandhak) mix *these in a mortar till all the finest particles of mercury disappear and to these* add 1 part of iron oxide obtained according to the Ayurvedic system by burning steel 100 times or less (This being difficult to obtain, people use ordinary Europe made iron oxide) This is made into scales

Give 2 grms of this morning and evening The patient is not allowed to take anything but milk, sugar is allowed with milk and some fruits like oranges and Kabuli pomegranates In a few days the patient's appetite becomes voracious, he works up to 10 seers* of milk *during the 24 hours but at the same time, he has several motions* up to 10 to 12 in the 24 hours In spite of these liquid motions, the patient gains weight and strength very rapidly He becomes red and gains considerably in weight They go on increasing the dose up to 15 days and then gradually decrease it to 2 grms twice

* 1 Seer = 32 Ozs (English)

a day Then they cease and come very gradually to a normal diet In the hot weather they give chalk in the place of milk.

The second method is by means of *Bhilama* a fruit which grows wild in the Nizam s territory They take out the oil of this fruit and start treatment with half minim doses and gradually increase it During this treatment they do not confine the patient to milk They allow a rich diet containing ghee sugar and wheat flour but they stop salt altogether There is one great drawback to this treatment *Bhilama* produces a good deal of cutaneous itching and a red eruption and in some cases the private parts swell up if the drug is pushed indiscriminately The patient's appetite increases greatly and he can digest 8 chittacks* of ghee per day If itching comes on they give cocoanut kernals by the mouth and cocoanut oil to rub on the part In some cases the cutaneous itching may come on to a certain extent every hot weather for some years or only for a few days

The third mode is by *Loha chooran* containing metallic mercury, sulphur and certain other ingredients They give chalk in this treatment

I myself got sprue while practising in Calcutta between 1910 and 1915 I left the place and got a good solid motion for the first time in the train near Lucknow I took nothing but milk for four months and then came to a solid grain diet very gradually Now I am very stout and can take hard exercise

Lieut Col R McCarrison I M S (British India) It had not been my intention to take part in this discussion having indeed but little to contribute to it But since Col Mackie has referred to certain experiments carried out by me in monkeys some 10 years ago, I may give here a few detail in regard to them The experiments were designed, not with the object of producing sprue but of determining the effect on the gastro intestinal tract of ill balanced food deficient in vitamins The animals were fed first on a diet of white rice, butter and water After periods ranging from 15 to 30 days two out of six monkeys fed on this diet developed a form of diarrhœa in which comparatively large amounts of pale-coloured frothy motions were passed suggestive of the stools in sprue On post mortem examination a profound alimentary dystrophy with gastric atony great thinning of the walls of the entire tract and intense degenerative changes in the mucous membrane of the tract were observed With these intestinal changes there were associated degenerative changes in the liver and pancreas and hæmorrhagic changes of a disruptive nature in the parathyroids The diet I used was one that had many defects deficiency of vitamins of the A B and C classes together with a lack of mineral elements and its want of balance in proteins fats and carbohydrates Col Mackie has referred to an experimental diet which I suggested to him for use in the work being done on sprue at the Haffkine Institute He wished me to suggest a diet in which the main deficiency was one of vitamin C This I did but the diet was one designed to produce an acute avitaminosis It may be that an ill balanced diet which gives rise to a more chronic state of avitaminosis would be more suitable for his purpose since on such a diet his animals would live longer

To me the chief interest in Col Mackie s paper lies not so much in its importance in regard to sprue but in his observation that the deficient diet which he used gave rise in his monkeys to well marked gastro intestinal lesions This observation originally

* 1 Chittack = 2 Ozs (English)

made by me in 1918, has now been so widely confirmed that it may be added to the list of the established facts of medical science and it is now to be recognized that one of the most important consequences of ill-balanced foods containing an *insufficiency* of vitamins and mineral elements is a profound disturbance of gastro intestinal function which may be the precursor of many gastro intestinal diseases I venture to think, therefore, that Col Mackie's interesting paper has a wider significance than in its relation to sprue

Lieut Col J Morison, I M S (Assam) In India, for at least thirty years, sprue has been held by many to be a concomitant of bacillary dysentery

Sprue has long been known in Bombay, Rangoon and in certain hill stations all places where dysentery and epidemic diarrhœa are common

In Poona, previous to 1914, sprue was of frequent occurrence The symptoms which we call sprue were described by Colonel (now Major General) J B Smith as forming part of graver sequelæ to the monsoon diarrhœa and dysentery which occurred at that place These diarrhœas and dysenteries were shown in 1914 to be mainly infections with the dysentery group Subsequent to 1916, with the abolition of the epidemic diarrhœa and dysentery in that place, sprue has become rare In September 1926, when the writer was trying Dr D'Herelle's bacteriophage in cases of bacillary dysentery, a lady was sent to him suffering with sprue She had been ill for two years and had the emaciation, the anæmia, the sore tongue and the persistent diarrhœa characteristic of that disease This lady very definitely dated the illness from an attack of dysentery There were no amœbæ in the stool which, in colour, consistence and quantity, was that commonly seen in sprue There was no dysentery bacteriophage in the stool and the association with dysenteries suggested a trial of bacteriophage in this case On the second day on which the phage was given, the patient was worse The tongue was more painful and the stools were more loose than usual We had agreed to a four days' trial before proceeding to more orthodox treatment Three days later the tongue was better than it had been for many months, the stools were solid for the first time for nearly two years, and ten days later the lady went on tour with her husband feeling better than she had felt since her initial attacks of dysentery This improvement was maintained until I left Bombay two months later and since then I have heard of no occurrence

Subsequent to this case, I have tried the phage in 22 cases Of these one, a hospital case in Rangoon, died Three have shown no improvement and 18 are definitely cured One case, ill for seven years, was restored to normal health in six weeks, having put on eleven pounds in weight Another, the worst case of sprue I have seen survive, was able to leave Rangoon in eight weeks for an up country station Another, an old lady of over sixty, I heard of a fortnight ago as very active and full of life

The full notes of these cases are being used by my colleague, Major Martin, for a thesis which I hope will soon see light but in nearly every case, by repeated examinations we have been able to isolate dysentery bacilli of the Shiga or Flexner groups It would therefore, seem that sprue is, in some cases, if not in all, really a sequela to an infection with dysentery bacilli and that it is amenable to Dr D'Herelle's treatment for that disease

Major S A White (U S A) · I believe that Col Ashford's contention that sprue is caused by *M psilosis* (Ashfordi) has not been proven, and Col Mackie's findings only confirm this belief

With regard to the claim made by some that sprue and pernicious anæmia are identical work done by Capt Fleming of our Army Medical Corps confirms the reader's finding that they are not Aside from the morphological pictures found in the blood in the two conditions, which differ as has been pointed out by Col Mackie the blood serum calcium also points to the conclusion that the two conditions differ essentially

In sprue (except during periods of remission or intermission when it may be normal) the serum calcium is uniformly below normal, while in pernicious anæmia even when severe it is not

I believe that Col Mackie's question as to the cause of sprue has been answered by Scott, and that sprue is the result of parathyroid failure, more particularly in its calcium control

Lieut Col J Taylor, I M S (Burma) Referring to Col Morison's remarks that sprue was less in Poona after the epidemic dysentery and diarrhœa was dealt with by the chlorination of the water supply in 1915, I find that taking the admission rates for dysentery and diarrhœa amongst British troops as an index of the prevalence of these diseases, the figures for the years 1910 to 1923 show that the disease was equally prevalent after 1915 and in some years considerably higher than before chlorination

ADMISSIONS RATES BRITISH TROOPS POONA

Dysentery and Diarrhœa

Year	1910	1911	1912	1913	1914	1915	1916	1917	1918	1919	1920	1921	1922	1923
Rate	38 3	41 0	31 1	32 7	11 5	50 8	13 3	20 7	32 3	55 1	43 2	64 5	43 2	27 3

(Chlorination)

Lieut Col J Morison, I M S (Assam) This is not the place nor is this the subject which allows me to place before you the full facts in connection with the epidemic diarrhœa and dysentery at Poona to which Col Taylor refers Suffice it to say that you will find the full report of the investigation in the *Indian Journal of Medical Research* for 1915 16 Further, I shall be glad to send to any interested the chart prepared not by me or even with my knowledge of the epidemic diarrhœa and dysentery in Poona for three years before and for three years after chlorination of the water supply This chart shows the complete abolition of the epidemic diarrhœa dysentery and cholera in the years after chlorination Moreover two years ago when some such remark as that made by Col Taylor came to my hearing I wrote to the Surgeon General of Bombay for the facts and received from him a letter and the actual deaths which showed that there had been no recurrence of the epidemic diarrhœa and dysentery which had formerly appeared every monsoon

Lieut Col F P Mackie I M S (Bombay) in reply Was glad to hear from Col McCarrison further information regarding the type of intestinal lesion met with in animals living on a vitamin C deficiency diet and agreed that his own findings confirmed these changes in a general sense Referring to Col Morison's remarks, he pointed out that the observations at the Haffkine Institute were definitely against the association of dysentery in sprue cases Col Morison brought forward the statement in support

of the supposed association that sprue was benefited by the administration of a dysentery bacteriophage but this fact might be explained equally well by saying that the bacteriophage was not specific

Replying to Major White he (Col Mackie) was most interested to hear that the American workers were also at one with him in denying the causative influence of yeasts in sprue The Haffkine Institute researches on the calcium content of the blood did not bear out the findings of Scott that there was a deficiency of ionic calcium in sprue or not at least in the majority of cases Even if, as Major White had argued, the causation of sprue was brought about by the bombardment of the parathyroid with toxic products, and the subsequent exhaustion of that gland, and the interference with calcium metabolism it still did not explain the origin of these toxic bodies and left the actual causation of sprue as mysterious as ever He did not accept the parathyroid hypothesis as the solution of the sprue problem

THE TREATMENT OF TROPICAL GASTRO INTESTINAL INFECTIONS.

BY

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Vice President, College of Physicians and Surgeons, Bombay , Member Bombay Medical Council , Late Medical Superintendent Arthur Road and Maratha Plague and Infectious Diseases Hospitals Bombay

THE extreme gravity, rapid course and a fatal termination of various acute gastro intestinal infections in the tropics are of sufficient importance to claim a passing notice It is proposed to confine this paper to but three of those, viz acute gastro enteritis of infants and children food poisoning and cholera No observations are required to illustrate these infections as they are so familiar to all I would therefore content myself with describing the line of treatment that I have adopted with marked and gratifying results

(*a*) *Acute gastro enteritis* in weakly and debilitated children is a disease of very rapid course and terminates fatally even within a few hours Prompt and efficacious treatment is necessary in order to stave off its progress and no line of treatment that I know of holds forth such promising success as the exhibition of minute doses of mercuric cyanide Although English medical literature scarcely alludes to the drug, it has been found to be a sheet anchor on the Continent e g in the Charite Hospital at Berlin and elsewhere in this affection as also in entero colitis enteric fever, etc Such minute doses from 1 100 grain to 1 50 grain (about 0 5 to 1 mg) administered every hour or even at shorter intervals act remarkably well in stopping the flux and in conducing to rapid recovery

(*b*) *Food poisoning* from milk, milk products and sweets, from meat cooked preserved or potted, fish fresh dried or preserved eggs and other sources not excluding over ripe and canned fruits and vegetables and other fresh vegetable irritants give rise to very threatening symptoms, including profuse vomiting and purging abdominal pain, prostration and collapse extreme restlessness hæmorrhages from the bowels, sudden menorrhagia and even abortion or miscarriage Sometimes hyperpyrexia has been observed And later, all the symptoms associated with the serious drain of fluid from the system such as cramps, faintness suppression of urine, dyspnœa, cyanosis and heart failure In fact, in the later stages, such cases are often mistaken for true cholera In these instances also mercuric cyanide has

a marvellous effect A few doses of 1/10 grain (6 5 mg) repeated half hourly or hourly stop the diarrhœa and the patients improve remarkably well within a few hours

Two of the most severe cases that came within my cognizance some years ago may be briefly summarized here In a Mahommedan family 12 persons one after noon had partaken of a sweet made from colostrum of a newly calved buffalo It was *freely mixed with various kinds of nuts* cardamoms nutmegs etc and treated with saffron Within four to six hours all of them became suddenly greatly prostrated with profuse and frequent watery evacuations vomiting etc One female miscarried at the seventh month and another had profuse untimely menstruation An elder member of the family exhibited the first signs whilst describing the history of the others In another instance also 12 persons in a Parsee family had partaken of cooked meat that had borne a journey of over 24 hours in a closed railway wagon They also developed grave symptoms with collapse within six hours In these series of cases recoveries were complete by the following day from the same line of treatment

Numerous *other cases have also been observed where fish eggs and vegetables* and fruits were concerned No fatality has however been observed in any of these cases in spite of the extremely threatening symptoms exhibited by the patients

(*c*) The drug has also been used in *cholera* in hospital as also private practice among nearly 4 000 patients during the period of over 20 years Its action in cholera it is not possible to surmize but exhibited early it controls both vomiting and diarrhœa and thus saves considerable after trouble which inevitably accom panies *the collapse stage The* evacuations *become smaller and less frequent and* there is re-appearance of bile in the stools Although a large majority of *the hospital patients were in the stage of collapse with evacuations the* late administration of the drug exhibited its effects It was exhibited in doses of 1/10 grain (6 5 mg) hourly or at longer intervals according to the number of evacuations as they became less frequent the frequency was reduced and thereafter the patients received about three times for three to four days

The drug is made up into mixture form —

Mercuric cyanide	1 gr	(0 065 grm)
Syrup simple	1 oz	(30 c cs)
Water ad	10 oz	(300 c cs)

Thus 1 oz represents a dose of grain 1/10 (6 5 mg)

It is not at all unusual for the first dose or two to be rejected but if persisted with and administered cooled subsequent doses are retained vomiting usually stops after one or two doses have been thus retained There is one drawback to its use however viz the development of stomatitis This depends a great deal upon the personal limit of tolerance Even one grain (0 065 grm) in divided doses as above within a few hours is well tolerated whereas in other cases even 2 doses

of 1/10 grain (6 5 mg) are rapidly followed by stomatitis Considering, however, the gravity of cholera stomatitis need not be considered a serious complication if it leads to ultimate recovery It might be well to add that the cases above related received no food, beyond black sweetened coffee without milk, plenty of water and ice or barley water No alcohol was administered under any circumstances Subcutaneous injections of camphor in oil, adrenaline, pituitrin, etc , were used as required Saline injections were not necessitated among the cases of enteritis and food poisoning though they had to be largely resorted to among advanced cholera patients

BACTERIOPHAGE

BACTERIOPHAGY AND BACTERIOPHAGE

BY

F D'HERELLE

In this short communication I shall not be able to dwell upon all the characteristics of the phenomenon of bacteriophagy I shall consider particularly the question so often discussed of the nature of this principle Since 1920 several hundreds of memoirs have been published on this question, moreover, the discussion has been conducted in a very peculiar manner Since 1917 I have shown that the characteristics of the phenomenon were only to be explained if the bacteriophage is considered to be a living organism, in 1923 I furnished a physiological proof of this which does not admit, I believe of any discussion Yet, none of the authors who proclaim *the enzymatic nature of the bacteriophage has ventured to explain all the characteristics* of the phenomenon on this basis, nor attempted to discuss the physiological proof which I have furnished As four years have elapsed since then I am justified in concluding that it is indisputable

At first sight it might be thought that the importance of this question is of a purely philosophical order, but such is not the case In reality, the whole question of the nature of ultra viruses is involved and it has its repercussions on the one hand upon the study of infectious diseases of plants and animals caused by these agents and on the other hand upon the problem of recovery from infectious diseases in general which as we shall explain in another communication, is not dependent upon a phenomenon of immunity, as hitherto believed but upon the behaviour of the bacteriophage

It is quite easy to isolate races of bacteriophages from the fæces of a patient suffering from an intestinal infection obtained at the time when the morbid symptoms are regressing At this time a bacteriophage capable of destroying and dissolving the pathogenic microbe, *in vitro* is found in the fæces

The bacteriophage passes through filter candles tight enough to retain all cultivable bacteria, it is sufficient then to emulsify carefully one cubic centimetre of fæces in a slightly alkaline medium the ordinary laboratory bouillon, and filter it through a Chamberland, Berkefeld or other suitable candle The bacteriophage will be found in the filtrate

Take a tube containing a young culture of cholera vibrios for example add to it one drop of the filtrate obtained from the stools of a convalescent cholera case After a variable interval of time depending upon the activity of the bacteriophage present, say, between 2 and 24 hours all the vibrios are dissolved and the medium is perfectly limpid

Add to a new tube of young culture a trace one millionth of a cubic centimetre of the clear liquid contained in the preceding tube after lysis is complete the same phenomenon of dissolution is reproduced and we can continue the series of passages indefinitely A double phenomenon is produced here destruction and dissolution of the vibrios and at the same time multiplication of the bacteriophages What was a culture of vibrios is now at the end of the process a culture of bacteriophages

Let us recall that the dissolving power of the various bacteriophages which can be isolated is extremely variable and it is not a question of quantity the billionth part of a cubic centimetre of a suspension of a powerful bacteriophage will be sufficient to obtain in a few hours the complete lysis of all the bacteria contained in 10 c cs of a young culture while 10 c cs of a suspension of a weak bacteriophage will only produce a partial dissolution indicated by a slight diminution in the opacity of the culture This partial diminution may only be momentary the medium becoming more and more turbid because the bacteria are capable of acquiring resistance against the bacteriophage In that case there is the formation of a phago resistant strain which develops in spite of the presence of the bacteriophage

The question of the resistance of bacteria to bacteriophage is extremely complicated Although it is of great interest I cannot deal with it here but it is sufficient to say that the phenomenon of bacterial mutations is for a very great part if not entirely dependent on it

The first question that occurs in considering the nature of the bacteriophage is that of its physical nature Does it exist in a state of solution in the liquids which contain it or in the form of granules in suspension? The first experiments that I carried out showed that the second hypothesis was the true one and that the size of the particles was equal to those of a micella of serum globulin This conclusion at first vigorously disputed is accepted to day by every author The diameter of the granules has been determined by various methods (ultra filtration optical methods), the most careful experiments fix their diameter at 20 to 35 millimicrons

Experiments carried out by Levaditi show that the corpuscles of vaccine virus herpes rabies and of bacteriophagy are all of the same diameter Recently Bechold *has succeeded in staining and observing bacteriophage as well as cow pox corpuscles* by the ultra microscope by means of a very delicate method consisting in first isolating the corpuscles from all other organic matter present and then precipitating colloidal silver in a condition of maximum dispersion on each corpuscle

The chemical nature of the bacteriophage corpuscle is still undetermined, in any case it does not seem to consist of a simple protein because it resists the action

of trypsin (which destroys for example bacterial toxins and anti toxins) It is probable that they are composed of nucleins

Finally what is its biological nature? The fact that it reproduces at the expense of living bacteria only permits of two hypotheses *either it is derived from* the bacterium itself and in that case is an enzyme or it is independent of the bacterium and so can only be a living autonomous being which utilizes bacterial substance in order to reproduce *No other hypothesis is logically possible*

Before any discussion took place I had in 1918 already considered the possibility of its enzymatic nature and had rejected this hypothesis because it could not *explain all the characteristics of the phenomenon for example the fact that* bacteria can acquire resistance to the action of a bacteriophage Consequently in the enzyme hypothesis it must be admitted that the bacterium responds to the *stimulus caused by a bacteriophage by creating a product identical with that which* caused the stimulus But we know that on the contrary living matter responds to any stimulus whatever by an antagonistic reaction which results in the production of an antibody whenever the phenomenon is possible and this is exactly what happens in the case of the bacterium in its struggle against the bacteriophage it reacts and acquires resistance The production of the bacteriophage by the bacterium and the resistance acquired against the bacteriophage are two incompatible phenomena Let us note besides that if the fact of the resistance of the bacterium is in favour of the living nature of the bacteriophage it is by no means a proof in the strict sense of the term We shall see in a moment what are the conditions which supply an absolute proof but before coming to this let us examine the principal argument of the supporters of the enzymatic nature of the bacteriophage It is this There exist in nature strains of bacteria which contain bacteriophages that can be isolated experimentally

Whenever an author puts forward an argument in favour of an hypothesis he should necessarily take as the basis of his reasoning a principle admittedly correct that is to say an axiom In saying There exist in nature strains of bacteria which contain bacteriophages therefore the bacteriophage is produced by the bacterium itself it seems to me that the axiom on which this depends can only be the *follow* ing — Whenever two principles are found together in nature one of them is pro

examples could be quoted Here is one that reproduces on a large scale the phenomena which occur in the interaction between bacterium and bacteriophage It is known that in tropical and even sub tropical regions all bovines are infected with *Piroplasma bigeminum* although their health in no way appears to be affected However *Piroplasma bigeminum* is a formidable parasite if it is introduced as has often been done amongst animals from uninfected temperate regions These animals contract piroplasmosis very rapidly after their arrival and die In the case of bovines of tropical regions there is a sym biosis—ox piroplasma—while in

the case of animals from uninfected regions, it gives rise to a fatal disease The accidental symbiosis, bacterium-bacteriophage, cannot therefore be considered as a pro[illegible] phage is produced by the bacterium for this phenomenon ma[illegible] Bacteriophage exists [illegible] ria which are habitual [illegible] nature to preclude us [illegible]

and this is equally true for [illegible] rd

Shall we say, as some have said, that the bacteriophage [illegible] too small to be living? Is life then a geometrical property? Certainly not and since it is not, a geometrical property cannot be used for measuring life Every phenomenon must be measured with its own particular standard life is a physiological property and it is to physiology that we must appeal physiological standards must be taken for measuring life, to know if a being is or is not living

The problem can be set forth in the following manner —Are there any particular characters common to all living beings to the exclusion of all others? What are these characters? Does the being under discussion possess these characters? If so, the question is irrevocably settled

The characters common to all living beings exclusively are (1) autonomy that is to say the possession of special individual characters differentiating it from all other living beings even those belonging to the same species (2) the power of chemical assimilation the faculty of transforming heterogeneous substances into homogeneous substances in harmony with the being that possesses this faculty and finally, (3) the power of adapting itself to surrounding conditions Such are the characters which together constitute the criterion of life

In the present state of science the proof of autonomy cannot be furnished for all beings considered as living Here is a striking example if the living nature of the Piroplasmata were in doubt if some author advanced that they were corpuscles resulting from an alteration of the cells of red cattle and explained the disease in a manner analogous to that employed by histologists in general to explain cancer it would be impossible to give any proof to the contrary because we could not demonstrate that what we call "piroplasma" is an autonomous being and consequently that it must assimilate in order to reproduce

Now, this proof of autonomy which cannot be furnished even for many organisms unanimously considered as living is possible where the bacteriophage is concerned

The fact that every race of bacteriophages which can be isolated presents peculiar characters, different from those of all other bacteriophages, is already

an indication of autonomy since uniformity is by contrast a general character of chemical bodies But it is possible to go further and by direct experiments to give an exact proof of autonomy I have furnished ten such proofs bringing different properties into play as a result of experiments carried out by me as well as by others

Here is one There exist races of bacteriophages which are active against a single strain of staphylococcus only and without action on all others On the other hand races of bacteriophages can be isolated which attack a very large number of different strains of staphylococcus Thus one race of bacteriophages which we shall designate by the letter *v* attacks only one strain of staphylococcus V, on the other hand the race *h* attacks a very large number of strains and amongst others the strain V It is evident that if the bacteriophage is derived from the bacterium if it is a bacterial product then the monovalent character of the bacteriophage *v* is derived from a special character of the strain of staphylococcus V at the expense of which it multiplies On the contrary if we can prove that this character of monovalence specifically belongs to this bacteriophage then it is independent of the bacterium It is easy to prove which of these two alternatives is true Let us make the polyvalent bacteriophage *h* multiply at the expense of the strain of staphylococcus V After a number of passages sufficient for all the corpuscles *h* inoculated into the first culture of V to be certainly eliminated by dilution say after 20 or 30 successive passages we prove that the bacteriophage *h* has completely retained its character of polyvalence Monovalence or polyvalence is thus a character belonging specifically to the bacteriophages and absolutely independent of the bacteria at the expense of which they reproduce The bacteriophage is therefore an autonomous being

Shall we say that the bacterium may perhaps reproduce a lytic enzyme of the same nature as that which produced the stimulus? Such a reaction would be strange indeed and contrary to all known biological facts and further the possibility of exalting the virulence of the bacteriophage by passages already shows that that is not the case But here is still a better proof

Recently Flu has isolated a race of bacteriophages active at the same time for the bacillus of Shiga and of Flexner *B coli* and *B pestis* He warms a suspension of this bacteriophage at a temperature of 58 degrees C and shows that the activity persists for *B pestis* and is entirely destroyed for *B coli* Flu then makes a large number of passages with cultures of *B pestis* and shows that the power to dissolve *B coli* is regained little by little Here then is a character 'Virulence for *B coli*' which is destroyed at a temperature of 58 degrees and reacquired by culture at the expense of *B pestis*

The illogical objection which might have been made to the first experiment can no longer even be invoked here

It is thus experimentally demonstrated that the bacteriophage possesses characters peculiar to itself and independent of the bacteria at the expense of which it multiplies It is therefore an autonomous being On the other hand since this independent being reproduces at the expense of bacteria which are foreign to it

it necessarily utilizes bacterial substance to secure its development and thus [illegible] by virtue of an act of assimilation

This autonomous being endowed with the power of assimilation is equally endowed with the power of adaptation I have indicated and the fact has been confirmed by various authors, that races of bacteriophages which cannot act in acid medium may become adapted, by a series of cultures in media of [illegible] pH, to produce total lysis in media of pH 5 8 where the same bacter[illegible] [illegible], is totally inactive I have adapted bacteriophages to the [illegible] of glycerine Prausnitz has succeeded in adaptation to phenol and [illegible] sublimate in such a manner that the adapted races survive in media [illegible] quantities of these antiseptics sufficient to destroy the same, [illegible] bacteriophages Asheshov has also carried out adaptation in [illegible] Prausnitz has succeeded in adaptation to anti bacteriophage serum.

These numerous experiments do not allow of any doubt of the [illegible] bacteriophage possesses the power of adaptation

Autonomy the power of assimilation and of adaptation [illegible] the criterion of life all beings which possess these characters, and the [illegible] is one of them, are undoubtedly living

The bacteriophage a living being is therefore an ultra [illegible] bacteria and provokes in them an extremely contagious [illegible] manifesting itself to us through the phenomenon of bacterio[illegible] communication we shall see the consequences of this on the [illegible] diseases in nature

THE PATHOLOGY AND EPIDEMIOLOGY OF INFECTIOUS DISEASES OF THE INTESTINAL TRACT AND OF CHOLERA IN PARTICULAR

I

BY

F. D'HERELLE,

MAJOR R. H. MALONE, I.M.S.,

AND

M. N. LAHIRI, M.B.

As one of us has already published on different occasions the observations and experiments carried out by him in various intestinal diseases, especially in bacillary dysentery, we shall consider more particularly the case of cholera in the present communication.

We have studied the pathology of cholera on patients treated at the Campbell Hospital, Calcutta, that is to say, in a region where cholera is endemic, and we have later on verified that the facts observed there recur in a similar manner in the case of persons attacked with cholera in the Punjab, where the disease exists in the epidemic form.

In the observations here recorded the gravity of the disease has been estimated each day in the following manner:—

We have applied a coefficient to each of the different symptoms, the sum of these coefficients representing the index of the gravity of the disease, the maximum being 10 for the most serious condition in which all the symptoms are present in a very high degree, the minimum 0, at the moment when convalescence is established.

Every day throughout the illness a specimen of stool was taken by one of us and carried as soon as possible to the laboratory. Immediately on arrival, a flake was spread on agar containing 0.5 per cent taurocholate of soda. The remainder of the specimen was added to peptone water placed in the incubator at 37 degrees until the next day, then filtered through paper covered with a layer of infusorial earth, then through a Chamberland filter candle L 3. After each filtration the candle was boiled in ordinary water, dried and heated to dull redness in a muffle furnace before being used again.

Each of the vibrios isolated has been completely identified.

When a patient died or was discharged as cured from the hospital, all of the filtrates were tested for the presence of bacteriophage and to determine the degree

of its virulence The test was carried out by allowing each of the filtrates to act upon a culture of vibrio isolated from the same specimen which had been used for obtaining the filtrate or, if the stools no longer contained vibrios upon the last vibrio isolated from the same patient

To 8 5 c cs of peptone water pH 7 8 to 8, was added 1 c c of a 24 hour culture of the vibrio in peptone water then 0 5 c c of the stool filtrate The tubes were examined after 2½ 5 and 24 hours The virulence of the bacteriophage was estimated in the following manner —

No action on the vibrio that is to say the turbidity produced by the culture being equal to that of a control culture without filtrate = 0 Total lysis after 2½ hours the tube remaining perfectly clear after 5 hours and 24 hours = 10 the maximum activity Between these two extremes stretches the whole scale of activity, that is to say all possible degrees of virulence of the bacteriophage For example, partial lysis after 2½ hours with total lysis after 5 hours the medium remaining perfectly clear after 24 hours = 9 No lysis after 2½ hours partial after 5 hours total in less than 24 hours = coefficient 8 etc We may add that in order to avoid any auto suggestion each of us carried out one part of the operations M N L the collection of the specimens and the observation of the patients in collaboration with the doctor in charge of the cholera ward R H M the isolation and identification of the vibrios and F D H the experiments relating to the bacteriophage Each noted in a separate register his observations and experiments and the three registers were only compared when all the researches, both in Calcutta and in the Punjab had been completed

Twenty three patients 7 of whom died had been studied in Calcutta 10 5 of whom died in the Punjab, that is 33 cases in all

The first specimens of each case were collected from 8 to 18 hours after the commencement of symptoms Of these 33 cases there were 12 deaths Six died within 24 hours of the onset and in none of these did we isolate bacteriophages virulent for the vibrios isolated from the stools of the patient himself or for any other strains of vibrios Two died between 24 and 48 hours after the first symptoms and in none of the three specimens obtained from either case during the course of the disease were there bacteriophages virulent for cholera vibrios

Finally, 4 died between 48 and 96 hours after the onset In these cases a bacteriophage of feeble virulence existed at the beginning but this feeble activity diminished and then disappeared The stools collected from 12 to 15 hours before death no longer contained bacteriophages virulent for cholera vibrios Thus in the 12 patients who died no bacteriophage virulent for the cholera vibrio existed in the intestine at the moment of death and in the case of those where it had been present at some time during the disease its activity remained very weak

In the case of the 21 patients who survived the behaviour of the bacteriophage was as follows —

In 5 a powerful bacteriophage existed in the stools at the first examination that is to say, from 8 to 18 hours after the first symptoms In each of these 5 cases

the morbid symptoms disappeared in the course of 24 hours and the patient was in full convalescence 48 hours after the onset It must be noted that these cases were not benign at the outset but were considered to be very seriously ill and amongst them was a woman 70 years of age

In the case of 16 other patients who recovered the increase of virulence of the intestinal bacteriophage with regard to the cholera vibrio manifested itself more slowly but in all of them *without exception* it reached a high potency between 24 and 72 hours after the commencement of symptoms and in all cases the favourable course of the disease was in correspondence with the increasing activity of the bacteriophage

We then set out to discover what was the behaviour of the bacteriophage in the midst of a community exposed to contagion rather than in a single individual

These observations were made in the Punjab in the district of Lahore from the beginning of June up to the end of August The commencement of the epidemic in this region dates from the 8th of May when cholera broke out in the little town of Kasur At the beginning of our researches the epidemic had spread to numerous villages in the neighbourhood of this town For lack of time we cannot describe in detail the observations and experiments carried out (these observations and experiments will be published later on in full in the *Indian Journal of Medical Research*) We shall give here only a brief summary

(1) Our researches show that in a region where an epidemic of cholera is raging there are a certain number of villages which we shall place in the first category *where no case of cholera previously existed* but which nevertheless are contaminated with non agglutinable vibrios and at the same time with bacteriophages virulent for agglutinable cholera vibrios These non agglutinable vibrios and bacteriophages can be isolated from the well waters and from the bodies of flies captured in the houses We have established that such villages appear to be immune even if they are in the neighbourhood of villages infected with cholera

(2) In other villages constituting a second category we have not been able to isolate bacteriophages virulent for cholera vibrios either from well waters or from the bodies of flies

(3) When the first cases of cholera develop in a village we have in no case been able to isolate bacteriophages from well waters or from flies These villages belong then to the second category just mentioned

(4) In the course of all the village epidemics studied there were never at the beginning any bacteriophages to be found in the environment but after a certain number of days, we were able to isolate both non agglutinable vibrios and bacteriophages virulent for cholera vibrios from well waters and from flies Thus villages which belonged at the beginning to the second category became during the course of the epidemic similar to those of the first category and it was from this moment that the epidemic was observed to decrease *pari passu* and finally to cease as ' contamination by the bacteriophage became generalized

From all these facts we conclude that the behaviour of the bacteriophage with *respect to the cholera vibrio is the same in the midst of a community in the course* of an epidemic as in an individual during the course of his disease In the case of an individual the onset the course and the final result of the disease depend upon the behaviour of the cholera vibrio and the intestinal bacteriophage towards one another In a community of susceptible individuals at the beginning there is a dissemination of the pathogenic vibrios proceeding from the first case introduced into the community, then a dissemination of bacteriophages proceeding from the first convalescent The beginning the course and the cessation of the epidemic depend upon the relative degree of contamination by pathogenic vibrios and by bacteriophages virulent for these vibrios, the variations of each acting upon the other in opposite directions

In cholera as in other infectious diseases of the intestinal tract the bacteriophage is the direct cause of recovery , the cure is contagious in the same respect as is the disease itself

The work here summarized has been carried out during the present year by means of a grant from the Indian Research Fund Association and we wish to tender our thanks to the Scientific Advisory Board of this Association and to its Secretary, Col J D Graham Our thanks are also due to the Superintendent of the Campbell Hospital Calcutta Col Acton acting Director of the Calcutta School of Tropical Medicine the Professor of Pathology Lahore Medical College and Col Forster, the Director of Public Health Punjab for providing many facilities for carrying out our researches

THE TREATMENT AND PROPHYLAXIS OF INFECTIOUS DISEAS
THE INTESTINAL TRACT AND OF CHOLERA IN PARTICULA

II

BY

F D'HERELLE,

MAJOR P H MALONE I M S,

AND

M N LAHIRI, M B

IN a previous communication we showed that in infectious diseases
intestinal tract the onset and the course of the morbid processes are inti
associated with the behaviour of the pathogenic bacterium and of the in
bacteriophage towards one another and that recovery is not caused, as h
accepted by a phenomenon of immunity, but indeed by the action of a b
phage whose virulence becomes exalted in the intestine of the patient and
the destruction of the pathogenic germs True immunity follows recov
which it is a consequence but does not precede it

It is evident that if recovery is due to the presence in the intestine of a b
phage of exalted virulence it would be sufficient to introduce into the di
tube of the patient a culture *in vitro* of bacteriophages highly virulent for the
genic bacterium in order to cause the destruction of the latter and the re
which results from this destruction provided always that this culture of b
phages has time to act before there are produced organic lesions sufficient to
death

We shall only consider here the treatment of acute diseases, and this is m
always the case where cholera is concerned, while it is not so in the dysen
southern India Burma and Malay where the chronic form is extremely fr
and in the great majority of cases the so called acute dysentery in the na
in reality only a relapse supervening during the course of a chronic dysentery
the behaviour of the bacterium and the bacteriophage with respect to one
is entirely different from what takes place in true acute dysentery For
time we cannot consider this question here

Since 1919 one of us has undertaken experiments in the treatment of b
dysentery by means of a single dose of 2 c cs of a culture of bacteriophag
all the cases treated, without exception the symptoms disappeared in the fo

24 hours These experiments have been published in a work which appeared in 1921

In 1923 similar experiments were carried out in Brazil at the Oswaldo Cruz Institute at Rio de Janeiro Twenty four serious cases of bacillary dysentery were treated by the administration *per os* of a dose of 2 c cs of a culture of bacteriophages In 22 patients morbid symptoms disappeared between 6 and 24 hours after the administration of the bacteriophage In two other cases, the same result was obtained in the 12 hours following the administration of a second dose given on the day after the first As a result of these experiments the treatment of bacillary dysentery by means of bacteriophage is generally used in Brazil where it now actually constitutes the routine treatment The Oswaldo Cruz Institute prepares a culture of bacteriophages which are sent out in sealed ampules and has discontinued the manufacture of anti dysenteric sera

At the end of 1925 a quantity of bacteriophage culture sufficient for the treatment of 100 cases was sent by one of us to the Sanitary Service of the Sudan The following passage from a letter from the Director of the Sanitary Service of the Sudan gives an account of the results obtained —

'The results of the treatment of bacillary dysentery with it have been little short of miraculous In every case, with the solitary exception of a child who was practically moribund when brought to hospital, the bacillary dysentery has cleared up within 24 hours'

Since then this method of treatment has been used in the hospitals of the Sudan

As far as the treatment of typhoid and the paratyphoid fevers is concerned a special difficulty exists which we cannot explain now owing to lack of time We shall simply say that since 1924 the question has been studied in Italy where treatment on a very large scale has been applied with excellent results according to Doctors Alexandrini and Doria who have undertaken these researches with the help of the Italian Government

Let us now come to the case of cholera As we have indicated in a previous communication, after having studied the behaviour of the intestinal bacteriophage in the course of the disease, we undertook experiments in treatment by means of cultures of the most powerful bacteriophages isolated during the course of the first researches

The experiments in treatment have all been carried out in various villages in the Punjab, the patients remaining in their homes without any special nursing In all the experiments in treatment and prophylaxis one of us (F D'H) prepared the cultures of the bacteriophages and examined the specimens for the presence of bacteriophages, the second (R H M) visited the villages, administered the treatment, observed the results and collected the specimens, the third (M N L) isolated and identified the vibrio In all the cases treated, the method of administration was as follows —

Two c cs of a culture of bacteriophages were mixed with about 10 c cs of water and swallowed by the patient in the presence of one of us (R H M)

Four c cs more were mixed with 40 or 50 c cs of water and left with the fa
with the instructions that the medicine should be swallowed by the patient
spoonful at a time during the next two or three hours In case the first dose wa
vomited within five minutes or so the first dose of 2 c cs was repeated No o
therapeutic measure was employed nor any special nursing, the patients remai
in their houses and being looked after by the family

In those cases where the patient was still seriously ill on the following day t
three doses of 2 c cs each were repeated

The cases treated by bacteriophage have not been selected It v
tered to all persons suffering from typical cholera at the time of the visit w
out taking account of the time elapsing since the onset of the disease and the
family agreed to employ this method of treatment only Thus it is that in o
statistics will be found a cas who was confined to bed with fever during the t
days preceding the first symptoms and another case to whom the bacteriopha
was administered 56 hours after the first symptoms although he had had suppre
of urine since the onset and was already in a condition of dyspnœa Neverthe
these cases have been recorded amongst the deaths, since they were treated in t
same manner as the others and in order that we should not incur the reproach
having selected our cases

On the other hand it is recognized that during epidemics one encounters
certain number of cases more or less benign where, in spite of the presence of ty
vibrios, the stools are not 'rice water' but simply diarrhœic We have trea
seven such cases all of whom rapidly recovered but we have not included th
in our statistics because one of the symptoms, 'rice water' diarrhœa w
lacking

We have taken as controls the cases present in the village on the day of th
visit who would not accept bacteriophage treatment as well as the cases occurri
in the same village on the day before and the day after those on which the expe
ments in treatment were made The control cases were treated some according
the methods of Hindu medicine others by a mixture of essential oils distrib
by the Government

Under these conditions out of 240 controls there were 143 deaths that is
mortality of 60 per cent which is the general mortality rate in the epidemics thro
out the Punjab

Amongst the 70 cases treated by bacteriophage, there were 6 deaths wh
a mortality of 8½ per cent The survival of the individuals who recovered
verified in all cases between 3 and 5 weeks after convalescence

The details of these experiments in treatment will be published at a later da

In a previous communication we stated the facts which led us to consider th
the cessation of cholera epidemics was due to the diffusion into the environment
principally by means of drinking water and flies, of bacteriophages virulent fo
cholera vibrios passed out with the stools of convalescents If this conclusion i
true it would be sufficient to spread in the environment cultures of bacteriophage

exalted virulence in such a manner as to assure their ingestion by the population in order to cause an epidemic in progress to come to an end

The interpretation of experiments in prophylaxis whatever may be the means employed is always extremely difficult because it is necessary to take into account the fact recognized by all but generally neglected that epidemics left alone and without any intervention on our part burn themselves out naturally after a more or less lengthy but sometimes very short period Under these circumstances for experiments in prophylaxis to have a definite signification it is necessary that they should be repeated a large number of times with concordant results and above all that they should always be instituted from the beginning of the epidemic Unfortunately in the Punjab it is only rarely that the sanitary authorities receive an early report of the outbreak of an epidemic in a village Let us add that another difficulty is present in the fact that generally the inhabitants attempt to evade or even to oppose prophylactic measures

Before explaining some of the experiments in prophylaxis which we were able to make we may note that in villages where patients are treated by cultures of bacteriophages of exalted virulence an experiment in prophylaxis is instituted at the same time for the exalted bacteriophages multiply in the intestines of convalescents are passed out with the stools and are disseminated into the environment as we have been able to verify on many occasions It is however evident that in these cases the diffusion of the exalted bacteriophages takes place more slowly than if the cultures are directly poured into the wells supplying drinking water and furthermore these bacteriophages are spread only in those regions of the villages where cases have been treated Here are the data relating to villages where experiments in treatment without other experiments in prophylaxis were carried out —

Asal Suleman 2 000 inhabitants Epidemic begins on the 31st 11 cases from the 2nd to the 6th Experiments in treatment on the of August A single case occurs afterwards, on the 9th

Dhalloke 1 000 inhabit t I pidemic begins on the 6 h of A from the 6th to the 11th I rriments in treatment on the 11 cases

Here now are experim t in three villages where treatm and at the same time cultur f bacteriophag s were poured in

Kot Anderson 800 inh it ts supplied by 5 wells of drinking breaks out on the 20th of A st From the 20th to the 24th cases of whom 9 die On the h afternoon 40 c cs of bacteri added to each of the 5 well N cases with 4 deaths occur from the 27th On enquiry it is di t that the principal well was on the 24th evening under th text of recovering a rug which fallen into it, and that the i tants hurriedly took in a stock of soon as this well filled up a On the 26th the wells are The epidemic definitely ceas the 27th of August

Ghang 1 500 inhabitants F rst case of cholera on the 16th the 16th to 18th, 32 cases occur th 15 deaths The village wells two of which furn h drink water Forty c cs of a culture are poured into each of these tw wells on the 18th and 19th of other wells, the water of which used for washing are treated of permanganate of potash to prevent the water from being Following this operation there are one benign case on the 19th the 20th, 3 cases of whom 2 are fatal on the 24th and one fatal case From an inquiry made that day it is discovered that a well yard of a house about 100 yards from the village was hidden visits were made and from this well the friends of the themselves with a stock of water This well is bacteriophaged August, and no case has been reported since

Narwar Village of about 2 000 inhabitants Supplied by breaks out on the 2nd of August By the 4th of August mid-day the first 6 having died The population seem alarmed and the prophylactic measures suggested Two of the principal well infected area are treated each with 30 c cs of bacteriophage others receive a strong dose of permanganate Two of the six cases treated by bacteriophage and the others, too, in all probability drink water from the treated wells All recover and no further

We hope that the results obtained in the course of the experi up the Governments of countries where cholera rages, to and on a large scale, more especially as th methods of treatment by

 ny inconven they can be

:om main centres on account of the length of time during which cultures phages can be conserved

is prophylaxis is principally concerned it should be actually indispensable, to institute experiments with the object of determining the *absolute* the relative efficacy of each of the different procedures recommended scale large enough to ensure that the conclusions drawn shall be e We should endeavour to make comparative experiments between the ethods of 'vaccination' by the subcutaneous or oral route on the one prophylaxis by bacteriophage on the other It is not sufficient in fact two or more methods with one another for even if the experiment shows e of one method with reference to another the absolute efficacy of each ıy be equal to 0 This is however the method of experimentation which adopted In the course of all the epidemics in India the existence of a greater or smaller number of villages is only known when the last case it does not seem that the morbidity the mortality and the duration of ic is sensibly different in those villages from what they are in villages isual prophylactic measures are applied In all cases are not the progress ssation of the epidemic entirely governed by natural agents ? It is of importance for us to know if this is so If the efficacy of all the methods ted as being equal to 0 it would be useless to apply any of them it would y to limit ourselves to measures of isolation which are certainly effica should avoid useless annoyance of the population and the expenditure rable sums of money which could better be employed in some other on the contrary, one of the methods appeared to be efficacious every ıld be concentrated upon it and we should then hope to be able defi ontrol epidemics of cholera

ı this spirit that we desire to see experiments carried out on a large scale y to measuring the relative and absolute value of prophylaxis in cholera of bacteriophage But we strongly desire to draw the attention of the er to the following point which is essential —

iophage acts by its virulence against the pathogenic bacterium and not s One can isolate in nature races of bacteriophage with very different activity some with very little activity others of such activity that an al trace of the culture added to a culture of vibrios produces in three or ı a total dissolution of all the germs present So it is *essential* for 's as much as for treatment, that the cultures of bacteriophages utilized endowed with maximum virulence and maximum activity We are races of this nature and shall be glad to give cultures to any one who sto have them

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Asal Suleman 2 000 inhabitants Epidemic begins on the 2[illegible] 11 cases from the 2nd to the 6th Experiments in treatment on the [illegible] of August A single case occurs afterwards, on the 9th

Dhallohe 1 000 inhabitants Epidemic begins on the 6th of A[illegible] from the 6th to the 11th Experiments in treatment on the 11th [illegible] cases

Here now are experiments in three villages where treatments and at the same time cultures of bacteriophages were poured into [illegible]

Kot Anderson 800 inhabitants, supplied by 5 wells of drinking wa[illegible] breaks out on the 20th of August From the 20th to the 24th [illegible] cases of whom 9 die On the [illegible] afternoon 10 c cs of bacterioph[illegible] added to each of the 5 wells [illegible] cases with 4 deaths occur from [illegible] 27th On enquiry it is di[illegible] that the principal well was [illegible] on the 24th evening under the pretext of recovering a ring which was fallen into it, and that the [illegible] inhabitants hurriedly took in a stock of [illegible] soon as this well filled up [illegible] On the 26th the wells are again [illegible] The epidemic definitely ce[illegible] on the 27th of August

Ghang 1 500 inhabitants First case of cholera on the 15th [illegible] the 16th to 18th, 32 cases occur with 15 deaths The village is [illegible] wells two of which furnish drinking water Forty c cs of a culture [illegible] are poured into each of these two wells on the 18th and 19th of A[illegible] other wells, the water of which is used for washing are treated [illegible] of permanganate of potash to prevent the water from being us[illegible] Following this operation there are one benign case on the 19th [illegible] the 20th, 3 cases of whom 2 are fatal on the 21th and one fatal case [illegible] From an inquiry made that day it is discovered that a well [illegible] yard of a house about 160 yards from the village was hid[illegible] visits were made and from this well the friends of the ow[illegible] themselves with a stock of water This well is bacteriophaged [illegible] August, and no case has been reported since

Narwar Village of about 2 000 inhabitants Supplied by [illegible] breaks out on the 2nd of August By the 4th of August mid-day [illegible] the first 6 having died The population seem alarmed and anxi[illegible] the prophylactic measures suggested Two of the principal wel[illegible] infected area are treated each with 20 c cs of bacteriophage [illegible] others receive a strong dose of permanganate Two of the six cases [illegible] treated by bacteriophage and the others, too, in all probability [illegible] drink water from the treated wells All recover and no furth[illegible]

We hope that the results obtained in the course of these exp[illegible] up the Governments of countries where cholera rages to [illegible] on a large scale, more especially as the methods of treatment and [illegible] by bacteriophage are extremely simple, do not cause any inconv[illegible] tion entail a minimum expenditure and finally, since they can b[illegible]

far away from main centres on account of the length of time during which cultures of bacteriophages can be conserved

As far as prophylaxis is principally concerned it should be actually indispensable, we think to institute experiments with the object of determining the *absolute* as well as the relative efficacy of each of the different procedures recommended, and on a scale large enough to ensure that the conclusions drawn shall be indisputable We should endeavour to make comparative experiments between the different methods of 'vaccination' by the subcutaneous or oral route on the one hand and prophylaxis by bacteriophage on the other It is not sufficient in fact to compare two or more methods with one another for even if the experiment shows us the value of one method with reference to another the absolute efficacy of each of them may be equal to 0 This is however the method of experimentation which is generally adopted In the course of all the epidemics in India the existence of cholera in a greater or smaller number of villages is only known when the last case occurs and it does not seem that the morbidity the mortality and the duration of the epidemic is sensibly different in those villages from what they are in villages where the usual prophylactic measures are applied In all cases are not the progress and the cessation of the epidemic entirely governed by natural agents? It is of the greatest importance for us to know if this is so If the efficacy of all the methods were admitted as being equal to 0 it would be useless to apply any of them it would be necessary to limit ourselves to measures of isolation which are certainly efficacious We should avoid useless annoyance of the population and the expenditure of considerable sums of money which could better be employed in some other way If on the contrary one of the methods appeared to be efficacious every effort should be concentrated upon it and we should then hope to be able definitely to control epidemics of cholera

It is in this spirit that we desire to see experiments carried out on a large scale with a view to measuring the relative and absolute value of prophylaxis in cholera by means of bacteriophage But we strongly desire to draw the attention of the experimenter to the following point which is essential —

Bacteriophage acts by its virulence against the pathogenic bacterium and not by its mass One can isolate in nature races of bacteriophage with very different degrees of activity some with very little activity others of such activity that an infinitesimal trace of the culture added to a culture of vibrios produces in three or four hours a total dissolution of all the germs present So it is *essential* for prophylaxis as much as for treatment that the cultures of bacteriophages utilized should be endowed with maximum virulence and maximum activity We are preserving races of this nature and shall be glad to give cultures to any one who would like to have them

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Dhalloke 1 000 inhabitants Epidemic begins on the 6th of August 14 cases from the 6th to the 11th Experiments in treatment on the 11th No further cases

Here now are experiments in three villages where treatment was carried out and at the same time cultures of bacteriophages were poured into wells —

Kot Anderson 800 inhabitants supplied by 5 wells of drinking water Cholera breaks out on the 20th of August From the 20th to the 24th are recorded 20 cases of whom 9 die On the 24th afternoon 40 c cs of bacteriophage culture are added to each of the 5 wells Nine cases with 4 deaths occur from the 25th to the 27th On enquiry it is discovered that the principal well was completely emptied on the 24th evening under the pretext of recovering a ring which was said to have fallen into it and that the inhabitants hurriedly took in a stock of pure water as soon as this well filled up again On the 26th the wells are again bacteriophaged The epidemic definitely ceases on the 27th of August

Ghang 1 500 inhabitants First case of cholera on the 16th August From the 16th to 18th 32 cases occur with 15 deaths The village is supplied by 4 public wells two of which furnish drinking water Forty c cs of a culture of bacteriophages are poured into each of these two wells on the 18th and 19th of August The two other wells the water of which is used for washing are treated with a strong dose of permanganate of potash to prevent the water from being used for drinking Following this operation there are one benign case on the 19th one fatal case on the 20th 3 cases of whom 2 are fatal on the 21th and one fatal case on the 25th From an inquiry made that day it is discovered that a well situated in the court yard of a house about 100 yards from the village was hidden when the previous visits were made and from this well the friends of the owner had provided themselves with a stock of water This well is bacteriophaged on the 25th of August and no case has been reported since

Naruar Village of about 2 000 inhabitants Supplied by 22 wells Cholera breaks out on the 2nd of August By the 4th of August mid day there are 12 cases the first 6 having died The population seem alarmed and anxious to submit to the prophylactic measures suggested Two of the principal wells situated in the infected area are treated each with 30 c cs of bacteriophage cultures All the others receive a strong dose of permanganate Two of the six cases still living are treated by bacteriophage and the others too in all probability since they must drink water from the treated wells All recover and no further cases occur

We hope that the results obtained in the course of these experiments will stir up the Governments of countries where cholera rages to undertake experiments on a large scale more especially as the methods of treatment and of prophylaxis by bacteriophage are extremely simple do not cause any inconvenience to the population entail a minimum expenditure and finally since they can be applied in regions

far away from main centres on account of the length of time during which cultures of bacteriophages can be conserved

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THE THERAPEUTIC USE OF BACTERIOPHAGE IN DYSENTERY IN RANGOON.

BY

LIEUT COL J MORISON, M B, D P H, I M S

AND

MAJOR C. DE C. MARTIN, M B, D T M & H, I M S

Pasteur Institute of Burma, Rangoon

A THERAPEUTIC test of bacteriophage is complicated not only by the difficulties *attending any clinical test in human beings, but by differences in the virulence of* individual strains of bacteriophage If one strain of bacteriophage be used throughout an experiment, the result of the experiment successful or not, is an attribute only of that strain

In December 1926, the use of bacteriophage for dysentery in Rangoon was begun with a strain from Bombay derived by subculture from one originally supplied by Dr D'Herelle This strain was active against our stock strains of Shiga and Flexner, both obtained from the National Collection at the Lister Institute, London, but we have persistently attempted to increase its valency During the process of examining 1,500 strains of bacteriophage from patients, when we secured a very active bacteriophage, especially from a case to whom no bacteriophage had been administered, this fresh strain, in most cases autogenous, was added to our therapeutic stock The bacteriophage which we now use is derived from the original strain, from four strains from cases that had received bacteriophage, and from twenty eight strains from cases to whom no bacteriophage had been administered In all we have used fifty six successive brews of bacteriophage, or over 5 000 doses, but we have no evidence to show whether the therapeutic value of the bacteriophage has improved by the addition of the newer strains

In another way, too, there has been a change At first the medium used was Martin's bouillon, as made at the Pasteur Institute, Paris This contained *ingredients which prevented the phage being used by a general Indian population*

We sought, therefore, to prepare a medium to which no religious objection could be taken

By the use of dried papaya juice and mutton, we have obtained a medium in which lysis takes place as well as in Martin's bouillon, and for the last six months all our therapeutic bacteriophage has been prepared with this medium

The clinical tests to which the bacteriophage has been put have been carried out on cases in private practice and in certain hospitals We have references to 58 cases in the care of our brother practitioners For private cases no controls were possible We can only say that those doctors who kept notes of their cases and sent samples of stools daily for examination have come more and more to rely on the early use of bacteriophage in dysentery A feature of the cases is the rapidity of the convalescence, and few recur

It seems specially valuable for Shiga and Flexner infections in children and in women during pregnancy or immediately after delivery Among the 58 cases there was only one death, and that occurred in a case of Shiga dysentery who made a rapid recovery under bacteriophage in Rangoon and at once started for England On the voyage home he had a relapse and died before getting to Marseilles This case had no bacteriophage given to him during his relapse

The hospitals in which the bacteriophage was tested were those of the Insein Central Jail, the Rangoon Central Jail and the Burma Oil Company at Syriam, the Rangoon General Hospital and the British Military Hospital In their relation to this test, these institutions fall into two groups —

Group 1 —Hospitals in which dysentery cases were treated strictly in turn, the first with bacteriophage alone and the next with whatever treatment—castor oil salines anti dysenteric serum, bismuth etc, which the physician considered appropriate The object was to compare the use of bacteriophage with the best available treatment.

Group 2 —Hospitals in which alternate treatments were not adopted and where the bacteriophage might or might not be the sole treatment for such cases as received the bacteriophage

Group 1 —The cases in this group were treated in the Rangoon Central Jail the Insein Central Jail and the Syriam Hospital by Major Flowerdew I M S, Major J Findlay, I M S, and Dr W E Crawford Each of these officers knew nothing of what was happening in hospitals other than his own and no control of the treatment was exerted from the laboratory These were three independent tests The controls and the test cases were treated alike as to diet and in discharging to duty or to a convalescent gang no discrimination was made The condition of the patient and the accommodation in the hospital were the determining factors The criteria for comparison are the number of days till the stools became free from blood and mucus and three or less in number, the total days spent in hospital and the mortality.

GROUP 1

TABLE I

Controls treated with saline anti dysenteric serum, or other remedy which the physician considered appropriate

Bacteriophage cases, treated only with bacteriophage

Controls

Hospital	Number of cases	Days till stools normal	Days in hospital	Deaths
Rangoon Jail	34	8 0	9 6	0
Insein Jail	8	6 7	7 3	0
Syriam	10	4 7	5 4	0
Total and averages	52	7 2	8 4	0

Bacteriophage Cases

Hospital	Number of cases	Days till stools normal	Days in hospital	Deaths
Rangoon Jail	36	6 3	7 8	0
Insein Jail	8	5 3	6 3	0
Syriam	10	4 5	7 1	0
Total and averages	54	5 8	7 4	0

Group 2 —In Group 2 are the cases at the Rangoon General Hospital and the British Military Hospital

The cases from the Rangoon General Hospital are all the cases of dysentery admitted from the 1st January, 1927 to the 15th September, 1927, excluding those in which amœbæ or cysts were found They were under the treatment of Lieut Col R Kelsall, V H S, D S O, I M S, Major J W Jones D S O, I M S and Dr Tha Doe Control cases did not alternate with the test cases receiving bacteriophage Sufficient bacteriophage made from papaya and mutton was not available till May and after that, bacteriophage was given to approximately one out of four cases We have included all cases of dysentery from the beginning of the year, for when, during July, we happened to take twenty one consecutive cases treated otherwise than with bacteriophage as controls to the cases treated during that month with bacteriophage we found that seven had died—an unusually high death rate for dysentery and obviously not suited to contrast with the lower mortality among the cases getting bacteriophage We consider that the control cases, extending over eight and a half months, give a fair approach to what may be called the

normal results of the treatment of dysentery at this hospital It must be borne in mind that these are disturbed by a proportion of cases who, seriously ill and not improving leave hospital If there is a prospect of a patient dying his friends may remove him against advice During the period the bacteriophage was being used 2 seriously ill cases out of 68 receiving phage and 13 out of 159 control cases left hospital in such circumstances

Eight cases moribund when admitted and dying within 48 hours, have been excluded Six of these would come into the class of controls and two had bacteriophage

Nor was bacteriophage alone used in the test cases Sometimes bacteriophage was given after castor oil, salines, anti-dysenteric serum or emetin had been tried without success, in other cases bacteriophage treatment was begun for one or two days and was changed before the effects of the bacteriophage could be fairly assessed

We understand and a study of all the hospital case sheets seems to show that the cases given bacteriophage were on admission not less severe than those treated otherwise

The test is unsatisfactory but it probably represents what may be expected where the treatment is tried with no sense of confidence for the first time in a large hospital As it stands, the evidence seems to show that the bacteriophage does not prejudice the prospects of the patients

GROUP 2

Rangoon General Hospital

	Number of cases	Days till stool normal	Days in hospital	Deaths	Percentage
Controls	233	8 7	11 6	28	12
Bacteriophage cases	68	7 0	11 3	8	11 8

TABLE II

British Military Hospital

	Number of cases	Days till stools normal	Days in hospital	Deaths
Controls	3	6	**	0
Bacteriophage cases	13	6 8	**	0

** All cases of dysentery were detained in hospital for six weeks or until three examinations of the stools for *B dysenteriae* gave negative results.

In the British Military Hospital the patients were under the care of Lieut-Col Meadows, D S O, R A M C, and Major Anthonisz, R A M C A scheme of controls was planned as in Group 1, but here—unfortunately from the statistical point of view—the first results with bacteriophage gave such a good impression that, subsequently, in addition to those given bacteriophage from the beginning bacteriophage was also given to the controls when improvement with other treatment was delayed It thus happens that the controls are 3 mild cases and the bacteriophage cases are 13, of which 7 were severe and 6 were mild.

TABLE III

Summary showing mortality in both groups

	Number of cases	Deaths	Percentage
Controls	285	28	9 8
Bacteriophage cases	135	8	5 9

Amœbic Dysentery.—Out of 266 cases of dysentery in the Rangoon General Hospital examined microscopically, 33 or 12 1 per cent had *Entamœba histolytica* or cysts, of whom 6 (18 8 per cent) died.

Clinical dysentery is recognized to have a variety of causes, not readily diagnosed during life and sometimes not even post mortem We have, therefore, extracted *from the above 120 cases all those which showed, on microscopic* examination, the cellular exudate of bacillary dysentery, or, on culture, *B dysenteriæ* (Shiga or Flexner) The results appear in Table V

TABLE IV

Cases in which the diagnosis of bacillary dysentery was made from the finding of B dysenteriæ *(Shiga or Flexner) or from the cellular exudate*

GROUP 1

Controls

Hospital	Number of cases	Days till stools normal	Days in hospital	Deaths	Percentage
Rangoon Jail	20	9	10 0	0	
Insein Jail	8	6 7	7 3	0	
Syriam	10	4 7	5 4	0	
Total and averages .	38	7 8	8 2	0	.

GROUP 1—*concld.*

Bacteriophage Cases

	Number of cases	Days till stools normal	Days in hospital	Deaths	Percentage
Rangoon Jail .	23	6·3	7·8	0	.
Insein Jail .	8	5·3	6·3	0	.
Syriam	10	4·5	7·1	0	.
Total and averages .	41	5·7	7·3	0	.

GROUP 2.

Rangoon General Hospital

Controls ..	39	9·2	13·1	7	17·9
Bacteriophage cases	27	7·5	12·4	4	14·1

British Military Hospital

Controls	3	6·0		0	
Bacteriophage cases	13	6·8		0	

TABLE V

Classification in accordance with laboratory findings

	B dys Shiga	*B dys* Flexner	Cellular exudate	TOTAL	Deaths	Percentage
Controls . .	33	14	33	80	7	8·7
Bacteriophage cases ..	38	10	33	81	4	4·9
TOTAL	71	24	62	157	11	7·0

CONCLUSIONS

Under the conditions of a controlled test not in all respects satisfactory treatment of bacillary dysentery by bacteriophage alone has been as effective as orthodox treatment given to control cases

In the Rangoon General Hospital a series not adequately controlled seems to indicate that the course of and the mortality from dysentery among cases treated with bacteriophage are not worse than in cases receiving anti dysenteric serum salines or other treatment appropriate to the cases

When consideration is limited to those cases diagnosed as bacillary dysentery by laboratory findings the results of treatment with bacteriophage appear to be definitely better than those with other treatments

We desire to thank Dr Forster, Dr Haynes, Dr Murray, Dr Patterson Dr Spence and Dr Taylor for testing bacteriophage in their private cases and for supplying notes and material for examination and we wish to thank the Medical Officers referred to in this paper for carrying out the treatment in the hospital wards under their care

DISCUSSION

Lieut Col R Kelsall I M S (Burma) I wish to offer a few remarks on Col Morison s paper regarding treatment with bacteriophage during a recent epidemic of dysentery in Rangoon First I regard Col Morison s statistics as quite unconvincing He has taken two criteria of comparison The number of days in hospital and the date on which the stools became fæcal Both these are very shifting points The number of days in hospital that is the date of discharge is dependent on many factors the desire of the patient to leave hospital or his desire to remain, or the demand for beds for urgent cases It may be said that in a very large series of cases such variations would be of little importance but the smallness of the numbers given by Col Morison does not allow of this Then again Col Morison has used as a basis of comparison all cases diagnosed as dysentery admitted to the Rangoon Central Hospital Such cases include all the cases which come in practically moribund suffering from terminal dysentery and are really cases—as shown by post mortem examination of chronic dysentery tubercle chronic nephritis etc etc Such cases cannot properly be used as a basis of comparison for dysentery mortality I have used bacteriophage in large numbers of cases of dysentery both acute and chronic and have used it in all cases working in conjunction with the Pasteur Institute It has as far as possible been used so that its effects could be compared with cases which were treated without bacteriophage After a very thorough and prolonged trial I have not been able to convince myself that bacteriophage has any therapeutic effect whatever in dysentery

I agree with Col Morison s remark, however, that 'Bacteriophage does not prejudice the prospects of the patient'

Dr A C Ukil (Bengal) Asked Dr D Herelle how soon and how completely the vibrios were dissolved in the intestines, for the speaker had been able to isolate

agglutinating vibrios from the stools of convalescents as many as 16 days after convalescence was fairly rapidly established The condition of the healthy 'carrier' condition and the convalescent 'carrier' condition required further elucidation It seemed that two kinds of lytic agents were involved in the fight against the vibrios—(1) those in the serum of the patient, which dissolved the vibrios in the presence of complement, and (2) the 'bacteriophage,' acting in the intestines We have to consider both these factors in understanding a rational therapy The question opened was a vast one and required further investigation, especially with regard to the question of continuing prophylaxis by anti cholera vaccines and other sanitary measures now being employed

Dr J N Das (Bihar & Orissa) About three years ago the public health department of Bihar & Orissa took up bacteriophage work Recently we had occasion to treat altogether 18 cases of cholera, two at Darbhanga and sixteen at Puri with bacteriophage alone Both the cases at Darbhanga recovered and of the 16 cases at Puri two died, thus the percentage of deaths among the 18 cases treated is only 11 per cent (as against Dr D'Herelle's about 9 per cent) These are certainly small figures to base a calculation upon, but the bacteriophage seems to open out a new line of treatment which may subsequently supersede the existing methods of treating cholera cases with drugs and salines

Dr J B Basu (Bengal) asked (1) How long this state of immunity by bacteriophage lasted in an individual that had once suffered from the disease and in whose intestines the presence of a corresponding bacteriophage had been demonstrated or could reasonably be inferred by the short course and comparative non severity of symptoms while the epidemic was at its height? The speaker asked this question because some cases of cholera had been known to relapse (2) Was immunity from cholera in healthy contacts during an epidemic due to bacteriophages in the intestine ingested or autogenous? If so, how were we to explain the passage of a very large number of Koch's vibrios amongst them and also among convalescents for 2 to 3 weeks?

Lieut Col J Morison I M S (Assam) replied In an investigation of this sort it is necessary to take criteria which shall as far as possible apply equally to the cases under special treatment and to the controls The criteria selected were the death rate the days in hospital, the days until the stools became fæcal No better have been suggested In the Rangoon General Hospital where Col Kelsall's cases were the deaths and the days in hospital are taken from the hospital records The condition of the stools is recorded on the case sheet by the nurse quite unaware of the use to be made of her notes To all cases, whether treated with bacteriophage or otherwise, these criteria were applied Every case sheet and chart as well as every post mortem record was scrutinized by Major Martin and myself These records are all available for further scrutiny if Col Kelsall so desires The fact remains that even in the unsatisfactory class including certain old standing debilitated cases that came into the Rangoon General Hospital the results conform with those of the more straightforward cases in the Jails in the Hospital of the Burma Oil Company and in the British Military Hospital

I may add that the whole of Col Kelsall's cases which form a fraction of the cases from the Rangoon General Hospital are dealt with in these figures and, if only he will scrutinize his own records, he will find the results as Major Martin and I have set

Of chemo therapeutic remedies the most generally adopted are the oils of the hydnocarpus chaulmoogra group and their preparations The methods of administering these are many—oral by inunction by the subcutaneous intramuscular *and intravenous routes After trying out all these we have found the intravenous* injection of the sodium salt one of the most simple and effective, and it certainly is most popular with patients *This method of administration which was first adopted* by Sir Leonard Rogers was given up because of the blocking of the veins which soon occurred but a new method by which the patient's blood is mixed with a 2 per cent solution of the salt in the syringe before injection has done away with this difficulty This method of administration is practically painless a very important matter when it is considered how long patients have to endure treatment

Another mode of treatment is to inject the pure sterile oil prepared from fresh seeds When the oil is fresh and carefully prepared it is not painful to any marked degree and patients stand it well Both the methods above mentioned are cheap *an advantage which is not inconsiderable when large numbers of poor patients* have to be treated

The ethyl esters, *generally given intramuscularly, have in our experience been* found more painful but equally effective They have the comparative disadvantage in a poor country like India of being more expensive

Other drugs used in leprosy are some of the heavy metals especially antimony and copper Much of the benefit observed from their use is probably of the nature of limiting and clearing up reactions, although there are indications that some copper preparations may be very useful in the destruction of the disease

Another drug which has a very important place in the treatment of *leprosy is* potassium iodide Fear of the reactions caused by excessive initial doses has for *long prevented this drug from being used effectively I shall only refer to it shortly* here as other papers dealing with its action have been prepared for this section

All the drugs referred to above appear to have some action either on the bacillus itself or what is more likely on the leproma with the result that the protective mechanism of the bacillus is removed and it is phagocytosed and destroyed

The second line of attack on Hansen's bacillus has been along the line of vaccines These have been prepared either by grinding up and suspending lepromatous tissue or by making suspensions of various *acid fast organisms which from* time to time have been supposed to be Hansen s bacillus under culture Frequently *valuable results have been* obtained by the injection of such suspensions but doubt exists whether this action is specific or of the nature of protein shock, as good results have also been obtained by injecting suspensions of tubercle bacilli specially prepared and even the injection of proteins such as milk, and drugs like turpentine which cause the breaking down of proteins in the body have given equally good and sometimes even better results

But when vaccine therapy is desired, in our experience the most effective form is the auto vaccination *caused by potassium iodide administered orally* The breaking down of leprous tissue, in some cases even by small doses of iodide gives us a

more effective and more easily administered and regulated form of vaccination than the injection of any vaccine

Counter irritation of skin lesions is another auxiliary form of treatment which cannot be neglected While we have found baths and friction in the sun beneficial we have obtained the best results by painting on a 1 in 3 solution of trichloracetic acid and by subcutaneous infiltration of skin lesions

As in all chronic diseases, the general health of the patient must be maintained The removal of accompanying and predisposing diseases the regulation of diet, exercise bowel and other sanitation, favourable hygienic and climatic conditions and most important of all a cheerful and hopeful mental outlook are details not one of which can be neglected in the fight against leprosy

A very important point in the treatment of leprosy is the study of each individual case Mass treatment will not give the best results Frequently improvement is rendered impossible by some careless habit or indulgence of the patient and these must be sought out and corrected if possible

With regard to the prevention of leprosy while forcible segregation may be effective in certain small isolated areas with a paternal or autocratic government such a method cannot be applied to India effectively except in a few cases

Two of the great stumbling blocks in the way of dealing with leprosy have been its supposed special connection with the anger of the gods and the supposition that it was irremediable These have driven patients to hide their ' taint as long as possible and have depressed them mentally and physically thus causing more rapid increase of the disease The declaration that leprosy is remediable and the placing of the means of remedy within the reach of all by training doctors and organizing treatment centres are likely to be the most effective means of prevention of leprosy in India The fact that within 19 days of opening a treatment centre in a rural area in the Bankura district 250 patients suffering from leprosy were attending and that once such a centre is opened and conducted by a suitable and well trained doctor the patients continue to attend though many of them have to walk 15 or 20 miles is one of the best proofs that could be desired that leprosy is remediable

For such centres we have found the iodide treatment the most effective and with this are combined small injections of hydnocarpus oil which render the treatment more active and please the patients who are disappointed if they do not get them

One great advantage of such centres is their comparative inexpensiveness as compared with the foundation of asylums and colonies and they are a much more effective means of reaching early cases They also serve as centres of propaganda and demonstrate the dangers of infection and the methods of avoiding it while patients as they recover prove to their associates the remediability of the disease

LEPROSY IN TRAVANCORE

BY

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FROM the Travancore census of 1921 it is seen that leprosy exists to a large extent in the State Out of a population of 4×10^6 there are 2 058 lepers (i e 0 005 per cent) This is double that of the rest of India According to the previous census the number was estimated to be 1,115 This points to an enormous increase in the spread of the disease during the last decade Even though such is the case the report of the Indian Leper Commission makes no mention of Travancore The general public and medical experts outside the State are thus apt to think that the disease is not prevalent to such an extent as to call for effective measures to prevent its spread In my opinion the figures given are decidedly under estimated Many people conceal the disease Others call it blood rheumatism and old advanced cases get labelled as ' leprosy Of the four administrative divisions the largest number of lepers are found in the central division (62 per 10^5) the northern division has 48 per 10^5) the southern division (13 per 10^5) and the high range division (2000 5000 ft) (3 per 10)

From the Map (*see* opposite) it will be seen that leprosy is more common along the coast line

At present there are three hospitals in the State where lepers are housed and treated Of these one is a Government Institution with accommodation for 232 lepers The other two are Missionary Institutions together accommodating about 100 lepers Dispensary treatment is carried on in three centres in the central division where the incidence is greatest (62 per 10)

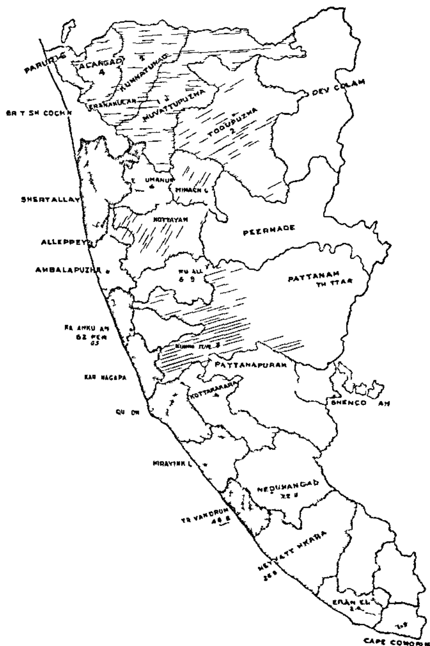
The regulation in Travancore regarding pauper lepers is very defective Only pauper lepers with visible sores on the body would be caught by the police and sent to the leper hospital There is no enforced segregation of lepers They may sometimes be found begging in the streets The lay public on the one hand have to be warned about the contagiousness of the disease On the other hand it is time that lepers realized that treatment in the initial stages offers reasonable prospect of complete recovery

Distribution of Leprosy —In Travancore the smallest number is found in the high range division Here the population consists mostly of estate coolies who are healthy adults under proper supervision The sanitation of the district is also

MAP OF TRAVANCORE

Showing Distribution of Leprosy

(Scale 9 miles to an inch)



good The elevation and the bracing atmosphere may also contribute to the low incidence of leprosy Next comes the southern division This has the least rainfall and the air is comparatively dry There is hardly any leprosy in the extreme south including Cape Comorin where there is only about 30 inches of rainfall per year and the air is very dry The Indian Leper Commission has also recognized that leprosy incidence is in inverse ratio to the dryness of the climate

The northern division has greater rainfall It has a greater leprosy incidence The largest number of lepers is found in the central division Here the climate is hot and moist and so favours the survival of the lepra bacillus outside the body as Rogers has suggested Largest incidence is in the coast line near the sea In this division there are lagoons back waters shallow canals sandy areas with stagnant pools and ponds in which cocoanut husk is soaked There are many breeding places of mosquitoes Sanitation is also unsatisfactory Overcrowding defective housing and poverty may also be other contributing factors

In Travancore leprosy and elephantiasis flourish side by side in sandy water logged tracts and it will be useful to investigate if *Culicidæ* have any part in the transmission of leprosy also Even though these two diseases exist side by side it is extremely rare for one individual to have both these diseases though a few cases have indeed been detected

1 *Factors favourable for spreading Leprosy* —Poverty with its results over crowding defective sanitation and deficient diet

2 Water logged sandy areas shallow pools and ponds in which cocoanut husk is soaked seem to be favourable soils for the spread of leprosy

3 Close association with lepers e g I know a particular instance in which a healthy adult contracted leprosy after living in the same house with a leper brother of his for about 20 years

4 Ignorance of the contagiousness of the disease

Ætiology

The Leper Commission said that ' leprosy cannot be considered a hereditary disease and that there is no inherited specific pre disposition to the disease by the offspring of leprous patients ' Later writers like Muir and Rogers have also come to the same conclusion In cases where leprosy has occurred in several members

(*i*) In nasal discharge when there is ulceration

(*ii*) From ulcers in other parts of the body, e g , in feet throat etc

(*iii*) In stools

(*iv*) In milk and semen

Predisposing causes —(*a*) Climate A climate that is hot and moist favours leprosy In central Travancore where the infection is very heavy rainfall is over 100 inches

(*b*) Age Statistics from State hospitals give maximal incidence between 20 and 40 This agrees with Rogers' figures

(*c*) Castes Largest number found among Hindu coolies. Higher castes are not exempt

(*d*) Sex The disease here as elsewhere attacks males more than females

(*e*) Diseases lowering vitality e g malaria, syphilis, yaws, etc

(*f*) Diseases of the gastro intestinal tract and intestinal parasites These are very common here

(*g*) Debilitating diseases e g , influenza typhoid etc

(*h*) Starvation This will contribute to a debilitated condition of the body

(*i*) Rat bite spider bite snake bite Nearly a third of the cases here give the above history Rat bite is the commonest It is supposed to be specially worse in July

Period of incubation —2 months to 2 years Average 2 to 4 years

Sites of initial lesions —In Trivandrum fingers of the hands leg toes abdomen breast and face are common sites for initial lesions

Varieties —Three (*a*) skin (*b*) nerve (*c*) mixed In Travancore nerve leprosy is the commonest The proportion of nerve leprosy to skin leprosy is as 4 1 or 5 1 Properly speaking there are only two varieties skin and nerve

Special reasons why nerve leprosy is common in Travancore The climate of Travancore is humid As a result rheumatism and various forms of neuritis are very common Diabetic neuritis is also not rare I myself am inclined to think that the so called anaesthetic leprosy is only a form of neuritis Anaesthetic patches can occasionally occur even in peripheral neuritis I have not detected lepra bacilli in the patches In the anaesthetic patch there is atrophy of skin glands as well as destruction of nerve endings In peripheral neuritis also the same changes occur The beneficial effects obtained by infiltration of ethyl esters of chaulmoogra may be due to the fact that the preparation being oily it stimulates the glands of the skin and improves its tone This must be the reason why other oils e g soya bean oil cod liver oil neem oil etc have also been found beneficial in anaesthetic cases The appearance of nodular leprosy is quite distinct from that of anaesthetic leprosy I have lately been trying intensive iodine treatment in leprosy I have found that this produces a violent reaction in nodular cases but there is no reaction at all in anaesthetic cases If both nodular and anaesthetic varieties are caused by one and the same germ one should naturally expect that a drug which reacts strongly in one variety would also act on the other variety This difference in reaction also upholds the view that these two forms are quite distinct and that the anaesthetic variety is more allied to neuritis than to leprosy proper If early anaesthetic cases be kept apart from nodular cases there would be very little chance of their getting infection The nodular cases have been proved to be very infective If we exclude the purely anaesthetic cases and concentrate our attention on the management and cure of nodular cases I venture to think that much would have been done towards stamping

out the disease In treatment by intensive iodine we have ready to hand a method by which quick results are found by experience likely to be obtained in nodular cases and by the same method also we can differentiate the so called anæsthetic cases from nodular leprosy.

Diagnosis

Cardinal points are—anæsthesia to light touch and finding lepra bacilli Besides these thickening of nerves want of sweating in special areas of skin and repeated febrile attacks may also be suspicious signs

Prognosis

Unfavourable as regards life From the Trivandrum Hospital reports it is seen that nerve leprosy cases live longest e g, one P Lakshmy has been in the leper hospital for the past twenty years I have come across two other patients in the same hospital who have been there for the past twenty years The nodular cases do not live for many years In our hospital there is one nodular case who has been there for the past eleven years

1 *Conditions influencing Prognosis* — Stage of the disease If treated early prognosis is good

2 Removal of exciting and favouring cause improves the prognosis

3 Natural body resistance and individuality of the patient are factors in the patient s favour

4 Dry temperate climate is favourable for arrest of the disease

5 Age Leprosy is not so common after 30

6 Chronicity of the disease Prognosis is good if the disease is not progress ing rapidly

Treatment

So far no specific for leprosy is known

The first essential is to improve the diet and the surroundings of the patient

Drug Treatment —It is well known that diseases caused by germs which are morphologically similar are considerably benefited by identical or analogous remedies As an illustration I may state that both syphilis and yaws which have been proved to be spirochætic infections are considerably benefited by injections of novarsenobillon and similar products The bacilli of leprosy and tuberculosis are observed to be quite similar in their appearance and staining reactions, the only difference being that the lepra bacilli are decolourized more easily than tubercle bacilli The lepra bacilli occur in clumps while the tubercle bacilli occur as separate rods Some years ago intensive iodine treatment was reported on favourably in cases of tuberculosis At that time I also tried that treatment in several tuberculous cases and got striking results in some cases As I found that the various methods of treatment of leprosy now in vogue were not quite satisfactory, I was led to give the intensive

iodine treatment a trial I selected half a dozen cases—4 nodular and 2 anæsthetic cases and started them on the treatment The following was the routine adopted —

At 7 a m each patient got 30 grains of potassium iodide dissolved in three ounces of water At 9 a m, 11 a m and 1 p m, he was given one ounce of chlorine water in seven ounces of water to which a little lime juice was added The chlorine had to be diluted so that it might be better tolerated by the stomach The object of the chlorine water is of course to get free nascent iodine This treatment was given for four nodular cases and two anæsthetic cases The nodular cases were all old cases which had not derived much benefit by injection treatment The four nodular cases got severe reaction—temperature going up to 102°—104° F The two anæsthetic cases had no rise of temperature This treatment has now been going on for a fortnight (i e from 13th to 27th September) In one case the reaction was so violent that I had to resort to adrenalin chloride to stop it and the medicine had to be discontinued for two days Already the nodular cases are showing improvement In one patient some of the nodules became swollen and have burst, forming ulcers These ulcers show signs of healing rapidly In another case the nodules have become softened The anæsthetic cases say that they experience a feeling of 'well being' due to the tonic action of the iodine Because of the severe reaction induced I expect to see rapid absorption of the nodules (*Photos of patients were shown illustrating the improvement effected in two and a half months by nascent iodine*) I think, in 'intensive iodine' we have a drug which will influence very favourably nodular leprosy As in the case of tuberculosis I am sanguine that by prolonged treatment with nascent iodine, the lepra bacilli in the body will be destroyed This has been proved to be the case by examination of smears before and after treatment for two and a half months The beneficial effect of iodine may probably be also due to the fact that in many cases of nodular leprosy there may be a past history of syphilis

(i) *Other Methods of Treatment* — Metallic preparations e g arsenic antimony and mercury I tried collosol antimony in a few cases Results were found to be poor I have no personal experience of arsenic or mercury

(ii) Sera and vaccines Sera are not successful

Vaccines —Non specific e g, typhoid and *B pyocyaneus* These have been reported to show improvement Sequeira thinks that the improvement is only due to protein shock

Nastin —I noticed slight improvement in anæsthetic cases

(iii) *Vegetable Oils and their derivatives* —Foremost is chaulmoogra oil extracted from *Taraktogenos Kurzii* This tree is observed to grow in the areas in Travancore where leprosy is endemic probably cultivated from early times as the oil had a reputation for curing the disease The seeds when dried can be chewed and eaten, starting with one-third of a seed thrice daily to one thrice daily The taste is not bad though some people may not stand it I have seen

improvement by eating seeds In villages patients are taking these nuts Now Dr Travers has advocated giving the powdered nut with *Cannabis indica* to prevent vomiting I have found if the nut be given in small doses there won t be vomiting Cochrane has had the best results with *hydnocarpus oil* with 4 per cent creosote This was tried in two grant in aid hospitals here with marked improvement in anæsthetic cases

Moogrol —I have found this very useful in nerve cases Both E C C O and E T O were tried in State hospitals

Results of treatment for 4 *years* —E C C O 384 patients received injection 5 discharged cured and 127 improved

E T O 559 treated 14 absolutelv free of symptoms and 193 improved considerably

I have been trying a mixture (sulphur and damor oil with 1 in 3 of chaulmoogra) in doses of three to fifteen minims Patients get relief as regards pains and muscular twitchings Colour of skin patches also shows improvement

I have also tried externally sulphur balsam (sulphur and damar oil) dissolving 1 part in 7 of cocoanut oil—this has given excellent results in leprotic ulcers The following is the system of treatment generally adopted by followers of the Indian indigenous system of medicine As a preliminary, they give emetics and purgatives This is followed by—

1 Chaulmoogra
2 Marking nut oil
3 Margosa

| Used both internally and externally (Rubbing with the oil and exposing to sunlight)

4 Oil extracted from python
5 Cupping for patches in the skin
6 *Venesection for advanced cases*

Concluding Observations

From the statistics I have already produced, it may be observed that central Travancore furnishes an excellent field for conducting researches into the treatment of leprosy It may be added that the occurence of elephantiasis and leprosy side by side is a tempting subject for investigation Different environments cause *different diseases but if these diseases happen to be infectious* they are naturally bound to affect more people even far away from that environment The study of environmental conditions on the spot, therefore, I venture to think is fraught with great possibilities for the future of medicine

NOTE SUR LE TRAITEMENT DE LA LEPRE

PAR

MAJOR V G F LABERNADIE
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A NOTRE arrivée a Pondichéry, nous avons utilisé comme nous l avions fait en Guyane Française (Amérique du Sud) les ethers éthyliques des acides gras de l huile de chaulmoogra en injections intra musculaires pour le traitement de la lèpre

Ils nous ont donne quelques résultats mais aussi quelques ennuis (*a*) L injection est quelquefois immédiatement suivie d une sorte de petit choc cardio pulmonaire (quintes de toux lipothymie) passager sans gravité mais désagréable pour le malade

(*b*) Le liquide injecté provoque quelquefois une induration intra musculaire douloureuse qui met une dizaine de jours à se resorber Au fur et à mesure du traitement ces noyaux deviennent de plus en plus nombreux il est fréquent qu une nouvelle injection arrive dans l epaisseur de ce tissu richement vascularisé penetre dans une veinule et provoque plus facilement encore le choc dont nous parlions cidessus Ces indurations sont moins frequentes et par conséquent les chocs lorsqu on utilise les éthers éthyliques *non iodes* qui entrainent une moindre réaction du tissu musculaire

(*c*) Nous n'avons pas observé d accidents graves en dehors des poussées aigues que certains auteurs considerent comme favorables à certaines époques de la maladie Il y a lieu dependant de remarquer qu une réaction intense parait mettre en danger la vie du malade comme nous l avons vu en Guyane

(*d*) Comme accident peu banal nous signalerons 2 cas de zona thoracique survenus au cours du traitement par les éthers l un avec les éthers éthyliques iodés (Guyane) l autre avec les éthers non iodés (Pondichéry) Nous avions dejà observe en Guyane le meme syndrome au cours d un traitement par l Eparseno

(*e*) Dans l ensemble le traitement par les éthers éthyliques non iodes nous a paru malgré ces incidents plutot favorable

* * *

Cependant les malades ne jugent pas toujours ainsi et ils reculent souvent devant les desagréments des éthers éthyliques iodés ou non iodes et abandonnent quelquefois le traitement — Aussi avons nous lu avec le plus grand intérêt l article de Muir(1) vantant l efficacité et la parfaite tolérabilité de l huile d hydnocarpus creosotée

Nous traitons ainsi une quinzaine de malades depuis Juin 1927 Les injections (traversée de la peau comprise) sont absolument indolores et ne provoquent les jours suivants aucune reaction locale Nous n avons jamais observe de choc cardio-pulmonaire

Les réactions focales qui se produisent quelquefois sont d intensité moyenne progressives et ne surprennent pas le therapeute qui peut les limiter ou au moins ne pas les aggraver Cette preparation nous parait efficace bien qu il soit trop tot pour nous prononcer En tout cas nous avons vu comme Muir des macules hyperemiques s attenuer des macules hypochromiques foncer des tubercules s affaisser des nevralgies se calmer

Etant donnes le faible prix de revient de cette drogue la facilite avec laquelle des malades supportent le traitement par injections cette methode merite de se generaliser

INDEX BIBLIOGRAPHIQUE

(1) Muir (1927) Comments on the present position of the treatment of leprosy *Ind Med Ga* April p 211

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ON THE CURATIVE VALUE OF THE TUBERCLE BACILLARY AUTOLYSATE IN LEPROSY

BY

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From the F D Petit Laboratory Byculla Bombay

In 1918 when the therapeutic and 'specific' qualities of morrhuates gynocardates etc , of soda and ethyl esters derived from the same or other sources was being prominently brought to the notice of the profession by their enthusiastic advocates as *the* remedies for tubercle and leprosy it was felt that if one could obtain similar compounds from the fatty and waxy acids of tubercle bacilli themselves such compounds might show even more potent and specific properties than the former derived as they are from drugs having no other claim than their time honoured reputation in these diseases In the solution of this problem *nothing was of greater value to the author than the application of some well* known physiological facts on enzymes in general and the specific nature of these bodies in certain cells in particular their specificity of action being purposive and depending on the richness of these cells in one or another of their constituent proximate principles Thus one would expect to find the lipase in a lump of tubercle bacilli (which is known to be rich in fatty and waxy substances) and, as a matter of fact it was demonstrated by Kendall Day and Walker to exist in solution in their broth cultures One can however more easily demonstrate the presence of these enzymes in a mass of tubercle bacilli grown on solid media when this is put up for autolysis after suitably treating it with chloroform or toluol which while killing the bacilli does not destroy the ferments

The results of autolysis are (*a*) a separation of fatty acids from the bacillary mass, and (*b*) conglutination of the residual bacilli with their altered staining and physiological properties

From the study of each of these when separated and purified one may summarize the following facts

(*a*) The fats and acids when separated yield a brownish waxy material soluble in fat solvents yielding an emulsion in water and colloidal suspensions when suitably saponified with alkali These however possess neither antigenic qualities when tested with the sera of infected animals nor any therapeutic properties when injected into such animals or patients on the contrary they set up such a

severe local inflammation as to cause the continuation of further observation impossible

(*b*) The bacillary part of the autolysate shows physical chemical and physiological alterations — (1) It is now free from acid fast characters (2) It forms an agglutinated mass which when dried and purified goes into a white powder yielding suspensions in saline solution (3) These show definite antigenic properties feeble in tuberculous sera but very strongly marked in leprous sera (4) They yield definite and beneficial results in a variety of tuberculous lesions when used as vaccines in definite doses (5) Their general application however in pulmonary tubercle is restricted by the limitation obtaining in this disease owing to a variety of uncontrollable social hygienic economic and other circumstances leading to rapid general asthenia wasting and cachexia (6) Hence the extension of its application in leprosy where the absence of the last mentioned bodily conditions hold out a better prospect of success especially in view of the more potent antigenic properties of the vaccine above referred to It is in this connection that the following observations are recorded The vaccine when injected subcutaneously produces hardly any general reaction even when the dose is gradually increased up to 0 1 milligram In 0 5 c c of saline solutions the effect produced is a hard subcutaneous nodule which gradually disappears in about 2 weeks The dose being small produces no inconvenience beyond the pain of the needle puncture The cases of leprosy which have come under the vaccine treatment with the tubercle bacillary autolysate are of all clinical types An account of these appears in Appendix I Here is a brief analysis —The cases are divisible into (A) asylum cases and private cases (B) cases according to the nature of the lesions

(A) (1) The asylum cases treated at the Acworth Leper Asylum Matunga Bombay for about 12 months with weekly injections did not give the striking results one would have liked to see partly because most of these were in a very advanced condition of the disease with frightful facial and bodily disfiguration extensive ulceration and atrophy A few however showed improvement in having the facial and other thickenings reduced by cicatrization and shrinkage and healing of ulcers Four of these asylum cases however being of shorter duration and perhaps showing only the cutaneous anæsthetic patches and nerve lesions some with and others without œdematous thickenings have done remarkably well and when last seen showed no retrogression even four months after stopping the treatment (*vide* Krishna Bhaskar and Swami) (2) The report of Col Kamat of the 8 cases treated at the Ratanagiri Leper Asylum however is very satisfactory This is given in Appendix II and will speak for itself (3) The report of Major Doyle of cases treated at the Yeiwada Leper Asylum which appears in Appendix I also shows satisfactory progress (4) The results of the cases studied by Dr J Oleviera Botelho are published with great enthusiasm in the *Journal Medical Nationale of Rio di Janeiro* and are highly flattering as to their therapeutic value But as the details are not available I am unable to append them here

(B) The best results are from the private patients, some under my own care and others under the care of my professional colleagues outside Bombay All of them have been able to come under my observations from time to time One can follow their after history The striking results are summarized in the conclusions and are probably due to the shorter duration of the disease and better social and economical conditions of these patients (*vide* Plate VI figs 3 and 4 and Plates VII to X figs 1 to 4 on each)

An analysis of the different clinical and pathological varieties and their response to the treatment herein indicated gives the following beneficial results in the order given below —

(1) The best results have been obtained in the cases with skin lesions e g anæsthetic patches of depigmentation with or without atrophy of the hair follicles and sweat glands with definite nerve origin but without much muscular atrophy

(2) Like those in (1) but with distinct subcutaneous thickenings with or without well defined raised margins looking reddish or with orange peel like skin and leonine appearance in the face

(3) Diffuse nodules or well localized soft nodules

(4) The most intractable are the thick fibrotic nodules with hard cartilaginous feel situated mostly in the ears and nose having either a corrugated thickened skin or thinned out shining skin giving a pearl white aspect with fine capillaries running over the surface

(5) Those cases showing great mutilation and hideous defacement and ulceration, probably from their long standing history and perhaps reduced vitality by a variety of complicating infections seem hopeless and beyond redemption as they cannot bear the injections well the local effects of the injections leading invariably to abscesses which are obstinate to heal even after their treatment surgically Some of these have been treated by a modified vaccine in large doses administered *per os* with some prospects of improvement

The *modus operandi* of the autolysate appears to be the stimulation and production in the system of a group antibody (in response to a group antigen) which acts quasi specifically on the cytoplasmic part of the acid fast bacillus causing their degeneration, death and subsequent absorption the waxy and fatty paraplasm being left to be disposed of by the tissues The hard nodule induced at the seat of injection is the result of such a local reaction where the *Bacilli lepræ* are mobilized from far to be subjected to the local destruction above indicated Such acid fast bacilli can be demonstrated in some clinically undoubted cases of leprosy where the bacilli in the nasal discharge have escaped detection This explanation of the action of the autolysate is different to that given by the advocates of morrhuates and other fatty salts who attribute the beneficial effects they obtain to the increased stimulation in the production of lipase which they claim has a better chance of acting on the acid fast paraplasm and exposing the bacilli thus rendered naked to the action of tissue fluids and cells In this connection it

may be of interest to refer to the investigations conducted by Golferken and Gharpure in my laboratory on the estimation of the lipase content of leprous sera as compared with that in non leprous sera These observers repeatedly found that the lipase in leprosy is not only increased but is 4 to 6 times that in non leprous sera a finding contradicting the statement of the advocates of morrhuates who found the lipase greatly reduced

Conclusions

The following résumé of the results of the action of tubercle bacillary autolysate when used as a curative vaccine in leprosy appears justifiable —

(1) The thickened nerve trunks become small and assume normal size

(2) The anæsthetic areas regain their sensation first to touch then to heat and cold and lastly to pain They become glossy and then resume their normal condition with the growth of hair and regeneration of sweat glands where these structures are involved in atrophy

(3) The colour of these atrophied skin areas remains slightly depigmented like the depigmented patches of pityriasis sometimes become over pigmented as if burnt away and sometimes resume the normal pigment especially in darker skins

(4) The margins of these areas when they are raised become flush with the surrounding skin and beyond a slight discoloration nothing abnormal can be noticed in these situations

(5) The trophic and perforating ulcers heal up rapidly, but in some cases the vulnerability of the parts remains very marked even after the healing up of the ulcers

(6) The atrophied muscles remain so and if at all recovering they are very slow in doing so and probably they remain as such if the muscular tissue has been destroyed by the disease

(7) In the cases with facial disfigurement the vaccine restores the natural contour of the features the thickened parts gradually melt away

(8) In the cases with tubercular nodules the vaccine leads to their absorption leading to crinkled up skin and return to the normal features provided these nodules are recent and not indurated

(9) A course of at least 25 injections one every week, seems to be necessary to show any definite changes in the gross lesions the dose being 0 025 to 0 05 milli gram or more gradually increased according to the patient s power of endurance

(10) In the cases of nodular lesions and especially when they are extensive and hard and when the skin is thickened and corrugated the vaccine seems to produce hardly any change even after a year s administration

(11) The first two or three injections may sometimes produce a mild focal reaction and make the lesions appear a little angry

REFERENCES

1 ROGERS L (1919) Proceedings, Indian Science Congress
2 ROW R (19 3) *Ind Jour Med Res* Vol. X Jan p C
3 *Idem* (19) Proceed s of the XIII Indian Science Congress p 1
4 *Id m* (19 3 24) Transact ons of the Grant College Medical Society
5 ROGERS L (1923) *I t Med Jour* Jul
6 ROW R (1924) *Ind Jour Med Res* Vol XI July p 19
7 *Idem* (19 4) *Br t Med Jour* December 13
8 *Idem* (19) *Ind Med P e* July
9 C LLEBKER and CHARPURE (1927) Proceedings, Indian Science Congress.

APPENDIX I

(A) ASYLUM CASES

(1) Cases treated at the Acworth Leper Asyl n Bombay

(An experiment extend ng over 16 months was carr ed o t by myself I commenced with 13 cases cons sting of anæsthet c and nodular variet es From t me to time some of the cases who had undergone treatment either used to abscond or refuse further treatment Whenever available I used t take on new cases from the new adm on By s the repeated adm ss ons and d scharges after 16 months work I found that I had not m re than 11 cases who had received treatment longer than 3 months However s ff ent opport n ty was afforded to draw con l s ons which have been embodied in the paper)

The foll wing cases I cons der worth report ng —

Raghu Balaj mal age 3 Anæsth s hands and feet and patches over the body S x months co rse f w ekly inject ons Sen b l ty to to ch h d returned p at hes ha l rega ned normal colour to some extent Seen four months aft r stopp ng the treat ent the mpro ement was not ced and no rel pse or rec rrence of f rmer symptoms was observed

Lama De j male age 3 Anæsthet var ety S x m nths co rse of weekly inject ns Se s b l ty to to h had ret rned S en fo r months after stopp ng the treatment the improvement was stea ly and there was no re rrence

Kr shna Bhaskar male a e 20 Bombay Anæsthes a and pat hes on the face and loss of ha r (Plate X fgs 1 and 2) S x months weekly i ject ons Anæsthes a d sappeared patches la e rega ned col r ha r has grown

Sonu mal a e 4 Bombay Ra sed red pat hes all ver the bod Three m nths weekly treatment All the patches replaced by flat pal spots

( ) Cases tre t d at the Patna Leper Asylum

(Ref No of 1927 S r D M L Leper As l m Patna 20th O t ber 19)

R M male age 3 Ulcerat s ea l ellow Ears th kened Cheeks e e brows ak n th kened and nodulated had coppery t nge F ers and toes th k ned and sw llen and had anæsthes a n both forearms from f ngers to elbows and fr m t es t b th knees (Plate X fgs 3 and 4)

Duration 8 years S xty seven nject ons fr m 20th February 19 6 Ulcerat n healed The former thi k nodules over the face and ears have ndergone considerable d m nut on the sk n ga n ng its n rmal appearance and c l ur Anæsthes a is remarkably decreased Superfi ial sensat on present n w to some exte t as it was bef re treatment

M R male age 4 Face odular w th coppery t nge ear nose and cheeks thickened F gers and toes intact Superf al se sation lost on anter r s face of the palms and soles Deep sensati ns lost in all the t es, s les of the feet dorsal aspect Durat on 9 years S xty three nje t ons from 20th February 1926 The nodules have d m n hed the ears and face re a n the r natural colour Anæsthes a not decreased Dep mented pat hes have all disappeared and reg n ed normal colour

may be of interest to refer to the investigations conducted by Gollerkeri and Gharpure in my laboratory on the estimation of the lipase content of leprous sera as compared with that in non leprous sera These observers repeatedly found that the lipase in leprosy is not only *increased but is 4 to 6 times that in* non leprous sera a finding contradicting the statement of the advocates of morrhuates who found the lipase greatly reduced

Conclusions

The following résumé of the results of the action of tubercle bacillary *autolysate when used as a curative vaccine in leprosy* appears justifiable —

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(6) The atrophied muscles remain so and if at all recovering they are very *slow in doing so and probably they remain as such if the muscular tissue* has been destroyed by the disease

(7) In the cases with facial disfigurement the vaccine restores the natural contour *of the features the thickened parts gradually melt away*

(8) In the cases with tubercular nodules the vaccine leads to their absorption leading to crinkled up skin and return to the normal features provided these nodules are recent and *not indurated*

(9) A course of at least 25 injections one every week seems to be necessary to show any definite changes in the gross lesions the dose being 0 025 to 0 05 milli gram or more gradually increased according to the *patient s power* of endurance

(10) In the cases of nodular lesions and especially when they are extensive and hard and when the skin is thickened and corrugated the vaccine seems to produce hardly any change even after a year s administration

(11) The first two or three injections may sometimes produce a mild focal reaction and make the lesions appear a little angry

REFERENCES

1 ROGERS I (1919) — Proceed n s Ind an Science Co re s
2 Row R (1923) — Ind Jour Med Res Vol X Jan p 69
3 Idem (1922) — Proceed n s of the XIth Ind an Science Con ress p 12
4 Idem (1923 24) — Transactions of the Grant Colle e Med cal S ety
5 ROGERS L (1923) — Brit Med Jo r July
6 Row R (1924) — Ind Jour Med Re Vol XI July p 105
7 Idem (1924) — Brt Med Jo r Decemb 13
8 Idem (1925) — Ind Med R e J ly
9 COLLEBKERI and CHARPURE (19) — Procee l ngs, Ind an S en e Congr ss

APPENDIX I

(A) ASYLUM CASES

(1) *Cases t eated at the Ackworth Lep r Asylu n Bombay*

(An exper ment extend ng over 16 months was carr ed out by myself I con nenced w th 13 *cases cons st ng of anæsthetic an l no l l r varieties From t me to t me son e of the cases who had* undergone treatment e ther used to abscon l or refuse further treatment Whenever ava lable I use l to take on new cases from the new adm ss ons By such repeate l adm ss ons and d scharges after 16 months work I found tl at I l ad not mo e than 11 cases wl o had receive l treatment longer than 3 months However suffic ent opport n ty was afforded to draw con lus ons wh h ha e been e n bod ed m the paper)

Tl e follow ng cases I cons der wortl report ng —

Pagh Baboj male age 35 Anæstl es a han ls an l feet an l patches over the bo ly S x months co se of weekly nject ons Sens b l ty to touch ha l returned patcl es ha l rega ned normal colour to some extent Seen fo r montl s after stopp ng the treatment the mproven ent was not ce l and no relapse or recurrence of f rn er sy nptoms was obser ed

Ra na D oj male age 3 Anæ thet c var et S x months cou e of we kl nject ons Sens b l ty to touch l ad returne l Seen four mo ths after stopp ng the treatment the mprovement was stea ly and there was no re n ren e

Ar shna Bhasl r n ale age 20 Bon ba Anæsthes a an l pat hes on the face an l loss of ha r (Plate X figs 1 and) S x months weekl ject ons Anæs he a d sappeare l patches l ave rega ne l colour ha r has grown

S am male age 46 Bombay Ra sed n l pat l es all o er the bo ly Three mo ths w ekly treatment All the patches replaced by flat pal spots

( ) *C es t eat d at the I atnag r Lep r Asylum*

(Ref No 2 of 19 7 S r D M P Leper As lum Patnag r 20th O tober 1927)

R M male age 32 Ulcerat ons o ea l elbow Fa s tl k ne l Cheeks eye brows sk n th ckene l an l nol lated ha l coppery t nge f n ers an l toes th kene l an l swoll n an l ha l anæsthes a n both forearms from f n e s to elbows and from toes to b tl knees (Plate X figs 3 an l 4)

Durat on 8 years S xty seven n ect ons from 20th F br ary 19 6 Ul erat on healed The former th ck nodules over the face and ears l ave n ler one con l rable l m n t on the sk n ga ng ts no mal appearance an l colo r Anæsthes a remarkably le reased Superfic al sensat on present now to son e extent as t was before treatment

M R n ale age 42 Face nodul r w tl coppery t nge ear nos an l cheeks th ckened fingers and toes ntact Superfic al sensat o lo t o anter or surfa e of the palms and sol s Deep sensat o s lost n all the toes s des of the f et lorsal aspect D rat on 9 years S xty three nject ons from 20th February 19 6 Tl e nodules have d n n l e l the ears and face rega n ng the r nat ral colo r Anæsthes not lecrea ed D p gmented pat l es have all d sappeared an l res med normal colour

N G, male, age 12 Face typically leonine, ears thickened, nose and cheeks thickened and nodulated Nodules on both thighs, outer aspect and on both calves All sensations present No wasting or ulceration present (Plate VI, figs 1 and 2) Sixty seven injections from 20th February, 1926 Thickening of the face and helix of the ear has wrinkled and the coppery tinge of the skin is fading and its place is replaced by natural dermis He also gets reaction His former leonine face has now transferred into monkey shaped with a wrinkling and squeezing of the nodules Sensation is intact

L G, female, age 32 Face lost its normal colour, became nodulated and thick, ears were also thickened Nodules appeared all over the body Superficial sensation lost in fingers and outer side of the right foot Sixty seven injections from 20th February, 1926 The face has undergone no change, it is still thick and nodulated Ears are thickened Sensation has not yet returned, there is diminution of the lesion

H A, female, age 14 Face and ears were first affected There were some nodulations and roughness of the face Sensation was intact Fingers and toes were intact Sixty seven injections from 20th February, 1926 The patient is fit to be discharged cured The nodules and roughness of the face has all disappeared and the face looks normal Microscopical examination of the skin and blood is negative

N A, male, age 72 Face no anæsthesia but a slight coppery discoloration wrinkling of the cheeks, ears thickened Fingers and toes normal All sensations except superficial ones are present Microscopical examination of blood from a leproma showed abundance of bacilli Posterior cervical glands are enlarged There are unripe cataracts in both eyes Fourteen injections from 19th May, 1927 There is no diminution in the thickening of the ears and coppery colour of the skin The anæsthesia is increasing and the numbness and deep sensation are not present

M A, male, age 12 Face slightly thick and rough, ears thickened skin had a peculiar colour and rugose and patchy No anæsthesia Fingers and toes intact Sixty seven injections from 20th February, 1926 Ears normal, skin normal, no anæsthesia There is still reddish tinge around the mouth The boy gets reaction after the injections and fresh crop of nodules appear which in course of time disappear Sensation is intact and fingers and toes intact

K H, male, age 14 Face showed slight roughness and a tinge of red hue Ears were enlarged and thick A circular depigmented patch 2 inches by 2 inches was on the top of the left shoulder All sensations present All fingers and toes intact No ulceration Sixty seven injections from 20th February, 1926 Roughness and redness of the face has disappeared The depigmented patch on the left shoulder has disappeared and the skin over the shoulder has assumed normal colour There is no lesion over the body except the thickening and enlargement of the ears which is stationary

A B, male, age 40 Ears thickened, no anæsthesia, fingers and toes intact, except a patch of anæsthesia on the lateral side of the right foot There was neither depigmentation nor ulceration nor hyperpigmentation There was thickening of the nerves namely, posterior auricular, ulnar and peroneal Twenty five injections from 14th April, 1927 Thickening of the ears lessened and general condition of the patient improved He now and then gets a reaction, but the lesions disappear very soon Thickening of the nerve is not lessened and the anæsthesia over the lateral side of the right foot is still present

B S, male, age 32 The skin of ears, nose, and cheeks is normal The bridge of nose is ... Fingers and toes intact The anæsthesia is ... It is all superficial except a patch 2 inches ... ion present Sixteen injections from 14th ... is much improved, for some three months the patient refused to have injections but now again he is receiving them

B T, male, age 27 A circular patch covering chin, lips, half of the nose and half of other cheek ... or aspect ... lost in ... he feet In right leg from knee downwards and the dorsum of the right foot ... injections from 20th February, 1926 The circular patch over the face is so diminished that it is scarcely

v s ble except on minute observat on The patch on the left chest reassum ng normal colour superficial sensat on and deep are sl ghtly diminished

R B male age 3? Face wr nkled but no anæsthesia and d scolorat on F ngers and toes d s torted The distal phalanx of right fingers and toes are ulcerated and wastin away All sensat on lost Twenty seven inject ons from 14th April 19?7 There is no impro ement and there is no d minut on of the anæsthes a area The sensation has not yet returned the general cond t on of the patient is good

G R female age 4? No d scolorat on and no anæsthesia over the face but was s mply in the forearm and legs Superfic al as well as deep sensat on was lost over the forearms and legs Ulcera t ons were present on the lateral s de of both legs Twenty seven inject ons from 14th April 19?7 Ulcerat ons healed soon after the commencement of the treatment but sensat on has not returned The anæsthes a s stat onary as t was before the commencement of the treatment

(3) *Cases t eated at th Yerwada Leper Asylu n Repo t f om Majo Doyle I M S Super nten dent Central Pr son Hosp tal Yerwada*

Reg ster No 6150 Name Ch ndhu Londhu Age ?3 years Sex male Address Y C Prison Occupat on conv ct, Y C Prison Durat on of d sease 3 years

Cond t on before treatment —Sk n over the eyebrows thickened Small nodules over the marg n of the p nna and lobules of both ears Sk n over malar eminences hands and lower half of both forearms is rough and cracked Sk n over lower extremit es to the junct on of lower an l upper half of thighs is also rough and cra ked Ulcerat on over the nasal septum Speaks w th a nasal twang Pos t ve to *Bac llus l præ* Treatment commenced 9th February 19?7 Total number of nject ons 19 Do e gradually ncreas ng from 0 5 c c to 1 c c weekly

Present cond t on —All that can be sa d n th s case is that the d sease l as not progressed The ulcerat on of the nasal septum has healed

Remarks —Developed cold ab ces es and fever hence treatment stopped

Register No 1018? Name Khudabux Ahmed Age 30 years Sex male Address Y C Pr son Occupat on conv ct Y C Prison Durat on of disease 7 months

Cond t on before treatment —Sk n o er the cheeks nose and eyel rows s th ckene l Small sh n ng nodular areas present on chest neck and back Sk n over the neck and f ont of chest is shin ng F ngers and toes are sl ghtly th kened R ght great toe s greatly th ckened Anæsthesia not present anywhere Ulnar ner es are th ckened Pos t ve to *Bac ll s lep æ* Treatment commen ed ?0th Ap l 19 7 Total number of nject o s ?1 Dose gradually ncreas ng from 0 5 c c to 1 c c weekly

Present cond t on —

Sk n over the nose cl eeks an l eyebrows	St ll th kened
Small sh n n nod lar areas	No change
I ngers and toes	No mprovement
Ulnar nerves	Just the same

Anæsthes a present over both insteps an l over both eyebrows an l cheeks

Rema ks —The disease has progress d n sp te of treatment, as ev denced l y the anæsthes a No inconven ence after nje t ons

Reg ster No 7?14 Nam Deolya Lakhya Age 30 years Sex male Ad lress Y C Pr son O cupat on conv t Y C Pr son Durat on of d ease 5 years

Cond t on before treatment —Skin over the forehea l eyebrows malar em nences alæ and t p of no e lower l p and chin s th kened an l presents d st nct nodules l art al anæsthes a over these parts Patches of th kened skin are present over the f ont an l back of the trunk Nodules are present over the pinnæ of both ears an l lobules are th ckened and there s complete anæsthesia Nodules are present over the ba k aspect of both forearms, front and external aspect of both arms Complete anæsthes a over extensor aspect only Thenar and hypothenar em nences are wasted F ngers are th kene l and na ls are undergo ng troph c changes There is compl te anæsthes a in both hands There are nodules over front and outer aspect of botl th ghs and o ter aspect of both legs Complete anæsthes a of both feet and legs up to the knees Patches of anæsthes a over the tl ghs Na ls of the toes are n lergo ng troph c changes Ulcerat on of the soft palate to wh ch the uv la is glued There is ulcerat on of the hard palate also Pos t ve to *Bac llus lepræ*

Treatment commenced 12th January, 1927 Total number of injections 18 of T B Vaccine Dose gradually increasing from 0 5 c c to 1 c c weekly

Present condition —

Forehead eyebrows malar eminences	Sensation improved and thickening lessened Nodules diminished in size
Nose and pinnæ of both ears	Sensation not improved Nodules diminished in size
Abdomen back chest	Improved in sensation
Lower part of both thighs Both arms and upper half of both forearms	Partially improved
Both hands and lower half of both forearms Both feet and lower half of both legs	Anæsthesia present
Nails of fingers and toes	Healthy nails have appeared
Nodules and thickening in general	Is lessened

Remarks —Very marked œdema all over body followed each injection with rise of temperature and rigors Cold abscesses supervened Hence treatment was discontinued after 18th injection

Register No 3952 Name Canu Daulata Age 30 years Sex male Address Y C Prison Occupation convict Y C Prison Duration of disease 4 years

Condition before treatment —Anæsthesia in the little finger and partial anæsthesia in the ulnar half of right and left forearms Complete anæsthesia over the right side of right chest Skin of the pinna and lobule of both ears thickened and skin over alæ of nose anæsthetic Malar eminences and eyebrows thickened and partially anæsthetic Both ulnar nerves thickened Positive to *Bacillus lepræ*

Treatment commenced 12th January 1927 Total number of injections 35 Dose gradually increasing from 0 5 c c to 1 c c weekly

Present condition —

Right little finger	Sensation has returned
Right side chest Inner side forearms Pinna and lobule of both ears alæ of nose and malar bones and eyebrows	Sensation not improved
Malar eminences and eyebrows Ulnar nerves	Still thickened

Remarks —No inconvenience after injections to the patient

Register No 8986 Name Tulasiram Dhanji Age, 40 years Sex male Address Y C Prison Occupation convict Y C Prison Duration of disease 2 years

Condition before treatment —Complete anæsthesia in both forearms and lower extremities below the junction of the upper and middle third of both the thighs Anæsthesia above the right nipple and over both scapulæ and inter scapular region. Lobules of both ears slightly thickened but are not anæsthetic and mottled in appearance Brownish patch near the nipple (right) and anæsthesia over the same area Positive to *Bacillus lepræ* Treatment commenced 12th January 1927 Total number of injections 35 Dose gradually increasing from 0 5 c c to 1 c c weekly

Present condition —

Forearms Lower extremities Above the right nipple Over scapulæ and inter scapular region	Sensation has returned
Lobules of ears	*Slightly smaller in size* and not anæsthetic
Brown patch near right nipple	Smaller in size and improved in sensation

Remarks —No inconvenience to the patient after injections

Register No 972 Name, Fulla Walla Age, 31 years Sex, male Address, Y C Prison Occupation convict Y C Prison Duration of disease 4½ years

Condition before treatment —Anæsthesia in both hands up to the wrists Wasting of thenar and hypothenar eminences both hands Diminished sensation in the left foot and a patch over the lower third of right leg (posterior aspect) Thickening of skin over both malar eminences Alæ of both nostrils thickened Both ulnar nerves are thickened Contractions of fingers and thumbs Positive to *Bacillus lepræ*

Treatment commenced 12th January 1927 Total number of injections, 35 Dose gradually increasing from 0 5 c c to 1 c c weekly

Present condition —

Both hands up to the wrists	No improvement in sensation
Left foot	
Right leg	
Ulnar nerves	Still thickened
Alæ and malar eminences	Thickening lessened.
Thenar and hypothenar eminences	Wasting still present
Fingers and thumbs	Still contracted

Remarks —Complains of pain after injections with a tight feeling in the hands Also has difficulty in breathing

Register No 7135 Name Sayad Abdul Sk Imambux Age, 35 years Sex, male Address Y C Prison Occupation, convict Y C Prison Duration of disease, 6 years

Condition before treatment —Thickening of the skin over the eyebrows Pimples over the nose and on the malar eminences Anæsthesia over the bridge and left side of the nose and right pinna Anæsthesia over the terminal phalanx of right ring finger A patch of anæsthesia over the dorsum of the right foot Positive to *Bacillus lepræ* Treatment commenced 12th January, 1927 Total number of injections, 34 Dose gradually increasing from 0 5 c c to 1 c c weekly

Present condition —

Eyebrows still thickened	Pimples on the face are present
Nose and pinna of right ear	Sensation has returned
Right ring finger	
Dorsum of right foot	

There is a general improvement in sensation Gross facial lesions not much altered

Remarks —No inconvenience to the patient after injections

Register No 65 Name, Bala Sakharam Age 60 years Sex male Address Y C Prison Occupation convict Y C Prison Duration of disease 10 years

Condition before treatment —Wasting of right thenar and hypothenar eminences Complete anæsthesia of right middle, little and ring fingers as well as palmar and dorsal aspect of right hand Tactile sensation much diminished in the right thumb and index finger as well as in the left hand Diminished sensation in the right foot and leg in its lower half Diminished sensation in the toes of left foot, with a patch over the middle and anterior aspect of left shin Positive to *Bacillus lepræ* Treatment commenced, 12th January 1927 Total number of injections 35 Dose gradually increasing from 0 5 c c to 1 c c weekly

Present condition —

Thenar and hypothenar eminences	Wasting is just the same
Left hand	Sensation improved
Right middle little and ring fingers and also palmar and dorsal aspect of right hand	Sensation unaltered
Right leg	
Left leg	Little toe is anæsthetic, others have improved

Patch over the middle and anterior aspect of left shin has disappeared and contracture of flexor tendons right hand not improved. No very marked improvement

Remarks —No inconvenience to the patient after injections

Register No 4885 Name, Vela Detla Age, 40 years Sex, male Address Y C Prison Occupation, convict Y C Prison Duration of disease, 15 years

Condition before treatment —Patches of brown pigmentation intervening with patches of lighter colour all over the body Partial anæsthesia over the brown patches and complete anæsthesia over lighter patches Wasting of thenar and hypothenar eminences of both hands more marked in the right than in the left Both ulnar nerves thickened Positive to *Bacillus lepræ*

Treatment commenced, 12th January, 1927 Total number of injections 35 Dose gradually increasing from 0 5 c c to 1 c c weekly

Present condition —

Patches of brown pigmentation and patches of lighter colour	Still present
Sensation over the patches	Not returned
Wasting of thenar and hypothenar eminences	No change
Ulnar nerves . .	Left thickened, right *slightly*

No improvement whatsoever

Remarks —No inconvenience to the patient after injections

Register No 8985 Name, Bhila Bhanaji Age, 30 years Sex, male Address, Y C Prison Occupation, convict, Y C Prison Duration of disease, 1 year

Condition before treatment.—Patches of discoloration with complete anæsthesia on the right eyebrow, right temple, left cheek and left malar eminence Similar patches on the ulnar aspect of both forearms and dorsum of left hand Similar big patches occupying the front aspect of the lower half of both thighs Similar patches on both calves Partial anæsthesia of both feet Positive to *Bacillus lepræ* Treatment commenced, 12th January, 1927 Total number of injections, 35 Dose gradually increasing from 0 5 c c to 1 c c weekly

Present condition —

Patches of discoloration on the body have disappeared all over except on both thighs

Right eyebrow, right temple, left cheek, left malar eminence and both calves	Sensation has returned
Forearms, dorsum of left hand and right thigh	Sensation partially returned
Left thigh and both feet	Still anæsthetic

Remarks —No inconvenience to the patient after injection

Register No 9715 Name, Ramchandra Ayappa Age, 40 years Sex, male Address Y C Prison Occupation, convict, Y C Prison Duration of disease, 3 years

Condition before treatment —Wasting of thenar and hypothenar eminences and interossei muscles of both hands Wasting of fingers which are contracted and flexed Complete anæsthesia in both hands Patches of brown pigmentation intervening with lighter patches on chest abdomen back and both thighs Partial anæsthesia in these regions Right ulnar nerve thickened Positive to *Bacillus lepræ* Treatment commenced, 12th January, 1927 Total number of injections, 18 Dose gradually increasing from 0 5 c c to 1 c c weekly

Present condition —No marked change Anæsthesia present as previously Pigmentation patches are less marked and fewer in number

Remarks —Fever with rigors and cold abscesses followed injections Hence treatment with T. B vaccine discontinued

(B) PRIVATE CASES UNDER MY OBSERVATION

Nodular

M, male, age 18 Nodules on the face and ears, and anæsthesia and paralysis right side ulnar nerve (Plate VI, figs 3 and 4)

This case has already been published in January 1926, and the patient has not received any treatment after that Seen in October 1927, there is no relapse or any advance of the disease

C, male, age 25 Nodules on hands, face and ear Weekly injections one year and a half

The nodules have flattened at many places, the facial expression has improved and the general condition is better (Plate VII, figs 1 and 2)

Miss W, female, age 40 Nodules on the ears, face and hands and destruction and distortion of features and limbs Weekly injections for one year and six months still continued

Changes in the nodules are evident features of the face, have recovered to some extent, formation of new nodules is stopped, general condition is much improved

M K, female, age 35, Navsari Nodules on the ears, thickening over eyebrows, patches of anæsthesia over forearm and leg (Plate VII, figs 3 and 4) Twenty injections from 29th September, 1926 up to 17th February, 1927

Patient looked very much better The nodules over the ears has almost disappeared Some thickenings at the spot had remained patches of anæsthesia were disappearing, ulcer on the toe had healed, no bandage or dressing was necessary; thickening over eyebrows was considerably less, almost imperceptible

G W K, male age 39, landlord, Khandala nandur, District Ahmednagar His face and fore head were thickly covered with nodules lobes of the ears, nose and eyebrows were all thickened. All these things had given the patient the characteristic leonine appearance There were many white patches spread over the chest, abdomen, back and hands Affected patches were insensitive to sensation Fingers were swollen and he used to get darting pains in them Fourteen injections from 22nd July, 1927 up to October 1927

Nodules on the face have disappeared but their places have been taken by reddish and blackish patches Insensitiveness of the affected parts has disappeared. White patches over the affected parts have disappeared White patches over the various parts have disappeared Darting pains over the fingers have completely stopped

Anæsthetic.

N Y K, male age 48, Jalgaon Anæsthetic patch about 3 inches in diameter over the right elbow one about quarter rupee in size, middle of the right thigh back sort of œdematous swelling below both eyelids and over eyebrows

The bigger patch is now much smoother, thinner, and of almost normal colour, but the edge is somewhat hypersensitive towards shoulder, a few hairs have now appeared the small patch over the thigh has regained sensation and colour œdematous patches below the lid appear to be the same, some improvement over the eyebrows is apparent General health much improved Twenty seven injections from 29th July, 1926 to October 1927

B H W, male, age 38 Bharanpur Lesion was an extensive area on the trunk and extremities. Fifty seven injections from 25th August 1926 to October 1927

The patient is getting better the lesion has decreased and sensation has been regained

P M, male, age 36, Aurangabad Anæsthesia over the extremities and patches over the front and back of the forearm, margins were raised patches œdematous itching and spreading Fourteen injections from the 4th January 1927 to October As no change was noticed, the treat ment was abandoned

B, male, age 40 Anæsthesia and patches on the eyebrows, below the lower eyelid and arm (Plate VIII, figs 1 to 4)

This case has already been published in January 1926, the patient has not received any treat ment after that Seen in August 1927 there is no relapse or any advance of the disease

M A P, male, age 40 Anæsthesia all four limbs Weekly injections from August 1923

The noticeable change in this case is that the symptoms getting blisters on the fingers remain absent as long as the injections are regularly given General condition is improved

F, male, age 40 This case has already been published in January 1926, the patient has not received any treatment after that Seen in October 1927, there is no relapse or any advance of the disease

Mrs V D, female, age 19 Anæsthesia and paralysis along ulnar distribution Weekly injections for six months

Perforating ulcer healed, anæsthesia reduced and muscles were regaining power, patient has not been seen since then

Mrs S C, female, age 22 Anæsthesia limbs and perforatin six *months* Anæsthesia was diminished, perforating ulcer healed

A D, male, age 40 Anæsthesia, patches on the body and injections six months (Plate IX, figs 1 and 2) Thickening of th regaining normal colour of the skin

M, male, age 16 Anæsthesia extremities and nodules on a year and a half *Anæsthesia is reduced and nodules are gradu*

I I J, male, age 16 Anæsthesia and patches all over the Weekly injections, one year, all patches regained normal co was stopped for four months, a few red patches on the body and recommenced and within a month all the patches and nodules dis

M, male, age 25 Anæsthesia and patches on the face, arm an Weekly injections for nine months Anæsthesia has disappea *normal colour*

K, male, age 35, anæsthetic Thickened red patch on the c Weekly injections one year

The red patch regained normal colour, hair has grown, progre

G G K, male, age 21, Bombay Thickening of all the finger four limbs Has had weekly injections from 18th August, 1926 anæsthesia has disappeared, general condition improved

S G G, female, age 50, Bombay *Patches on the back and f* widespread (Plate X, figs 1 to 4) Has had weekly injections fron has disappeared, patches have changed their colour to normal

Suleman, male, age 14, Bombay Ulnar anæsthesia, patches injections for one year Anæsthesia has disappeared, patches hav hair is growing

S I J, male, age 17, Dharwar The patches which were very and forearms have faded, leaving behind only white patch Sensati those patches which were quite anæsthetic before On the whole Sixty injections from *23rd June, 1925 to October 1927.*

G B I, male, age 30, Chandur, Berar —Both hands were sw below the left knee joint It was shining and slightly sensitive; u thicker than the right There was inflammation of both the nostril *12th November, 1926 Swelling of the hands has considerably go* marked over the patches General condition is good

M G P, male, age 24, Barsi Three small and two large patches colour, bright in appearance *There was no sensibility when pricked* April, 1927 No new patches Out of the five patches on the left s their sizes have been less, he feels sensation more at the borders but i

R R M, male, age 30, Khanapur, Morsi There was hot sensat brow and above the left forearm, a little above the left wrist, there heat and cold Seventeen injections from January 1927 There is commencement of treatment

D H A, female, age 26, *Sholapur* Anæsthesia on the ulnar dis ment from 30th October, 1927

P K G, male, age 50, *Sholapur Perforating ulcers* Eighty in 1926 The patient is getting worse and weaker

Mrs T J, female, age 32, Bombay *Anæsthesia upper and* ulcer Thirty six injections from August 1926 Anæsthesia is less,



Fig 1 Before treatment a large sized patch on face and marked thickening of the great auricular nerve



Fig 2 After 9 months treatment



Fig 3 Before treatment



Fig 4 After 8 months treatment

PLATE VI.



Fig. 1. Before treatment



Fig. 2. After 15 months' treatment



Fig. 3. Before treatment.



Fig. 4. After 4 months' treatment.



Fig 1 Before treatment



Fig 2 After 9 months treatment



Fig 3 Before treatment.



Fig 4 After 5 months treatment.

PLATE X

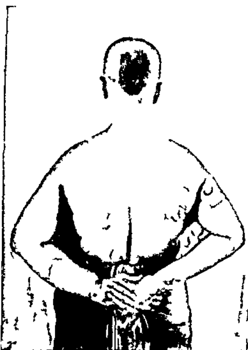


Fig 1 Before treatment Patches marked on back and extremities



Fig 2 After 6 months treatment



Fig 3 Before treatment Showing face



Fig 4 After 6 months treatment

APPENDIX II

RESULTS OF THE LATEST DRUGS ON LEPROTIC CASES TREATED AT SIR D M P LEPER ASYLUM, RATNAGIRI

BY

LIEUT COL D D KAMAT I M S

AND

V Y RANADIVE

SINCE the year 1922, we have been trying different types of the latest medicines on cases in our asylum Some of them were found very efficacious particularly in improving the ailments of the malady It cannot be said for certain however that they will effect a complete cure

The drugs which were used were sodium morrhuate ethyl esters of chaulmoogra and *Hydnocarpus wightiana* esters of linseed and margosic and lastly a vaccine prepared by Dr Row of Bombay

Among the preparations the esters of hydnocarpus and the vaccine gave us very encouraging results In 1922 we selected 5 cases for treatment with sodium morrhuate It was given twice a week subcutaneously in 1 c c doses One case of the nodular type was completely cured to all appearance and hence was discharged on parole He however came back in a worse condition in 1926 The remaining four showed no change

Next year, that is in 1923 we took for trial ester of chaulmoogra (I C C O) and the linseed ester prepared by Messrs Smith Stanistreet of Calcutta Eight cases were taken up All of them showed some improvement in one way or the other That is ulcers were healed up in some eczematous patches disappeared in others These changes were visible after 24 injections twice in a week The injections were continued for one year Beyond the changes already mentioned no improvement was marked and hence it was stopped Linseed esters were quite ineffective

In the year 1924 another preparation named hydnastryl manufactured by the same company was tried with a hope that it might give better results The same patients were again taken up It acted favourably and further improvement was marked I give below the five cases —

(1) *Laxmi Gena* —Hindoo female age 35 worst case nodules all over body ulcers on toes fingers, legs, forearms complete anaesthesia of both arms and legs and partial over her face Under treatment nodules decreased in size and number ulcers healed skin became thinner and sensation returned In all she got 200 injections

(2) *Govind Teli* —Hindoo male age 30 worst case ulcers on toes, fingers bleeding from the nose ears thickened anaesthesia of legs and forearms After 200 injections body became smooth Skin almost normal. Anaesthesia regained in legs and forearms

(3) *Hawabi* —Mohammedan girl age 10, nodules on face, ears thickened. After 200 injections nodules almost disappeared

(4) *Mohamed Ahmed* —Mohammedan boy, age 11, nodules on face, ears thickened Improvement was little

(5) *Kazi Hussan* —Mohammedan boy, age 13, nodules on cheeks, ears thick and long After 200 injections two or three nodules remained with a red tinge on cheeks

Whatever improvement was seen was effected in one year's time In the next year they all remained stationary in spite of the fact that the dose was taken to its maximum, i e , 12 c cs These large doses were causing inconvenience, discomfort and pain and drug was not absorbed in a week's time Besides, the drug was no more effective and the skin and subcutaneous tissues of the parts, selected for injections, became so thick that a prick with an ordinary needle became difficult and the tissue began to give way All these led to an abhorrence of the treatment and ultimately we had to discontinue it

By this time, I had read about Dr Row's successful vaccine treatment and we at once wrote to him to send us the drug for trial in our asylum

We took in all 8 cases Four were old ones, already treated with L C C O hydnastryl, 4 cases were new Out of the 8 cases, the following 3 were typically improved in one year's treatment

(1) *Mahadeo Rama* —Hindoo male, age 40 face, nose, cheeks full of nodules skin thickened, glossy, coppery tinge, sensations lost Under one year's treatment, nodules disappeared skin assumed normal colour and thickness, coppery tinge vanished along with glossiness, sensations regained

(2) *Bhiku Tukrae* —Hindoo male age 25, depigmented patch on nose spreading on either cheek giving the appearance of a butterfly Under treatment, line of demarcation practically invisible patches assumed brownish colour Wrinkling of the skin and sensations regained in depigmented area

(3) *Narayan Gopal* —Hindoo boy, age 12 big thick nose, cheeks rough with nodules, ears large and thick nodules on thighs and calves Under treatment, nose assumed normal size nodules on cheeks completely disappeared presenting wrinkled appearance The skin presents wrinkled appearance when it loses tenseness as a result of absorption of nodules

The other five cases gave encouraging results by absorption of nodules, and disappearance of coppery tinge which had not so far yielded to E C C O of hydnastryl

I am much impressed with this vaccine treatment because it has brought about further improvement in cases which were either stationary or showed little improvement with other drugs, specially E C C O and hydnastryl It has also an advantage over other treatments E C C O and hydnastryl require to be given subcutaneously twice in a week The maximum dose is 12 c cs , and must be continued at least two years, while the vaccine is given subcutaneously once in a week Its maximum dose is 2½ or 3 c cs at the most The course is of one year The big doses of E C C O and hydnastryl cause great discomfort, pain and inconvenience to patients as it is not absorbed readily All these disadvantages combined with the fact that the duration of treatment is two years make the patients reluctant to undergo treatment

On the other hand the small dose of vaccine is readily absorbed and the period is only one year The cost of 100 c cs of both hydnastryl and vaccine is the same, i e , Rs 4, but in the long run as the doses of hydnastryl are increased, the quantity consumed is greater and naturally it costs more Both the drugs are worth giving a trial and we wish that every leper institute would take the benefit of them

We are much indebted to Dr E Muir of Calcutta and Dr Row of Bombay for giving us a free supply of these drugs and valuable advice whenever sought for

THE IODIDE TREATMENT OF LEPROSY, WITH SPECIAL REFERENCE TO THE USE OF THE SEDIMENTATION TEST

BY

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I Iodide Treatment in Leprosy

Since *the time of Danielssen and long before the discovery of Hansen s bacillus* the remarkable effect of iodides in leprosy has been known but most writers have considered their action harmful because of the reactions produced these were supposed to indicate an exacerbation or reactivation of the disease Danielssen himself used it in apparently cured cases and if no eruption developed the cure was considered complete

The question of the nature of the leprous reaction produced by potassium iodide has been considered in another paper In the writer's opinion the exacerbation or reactivation produced is apparent and not actual provided that the administration is gradual and continuous If only one dose sufficient to cause a marked reaction be administered and the treatment be then stopped an increase of the disease is likely to take place But if potassium iodide is given in small doses to begin with and the quantity is gradually increased till a small reaction is produced and then continued once a week increasing according to the tolerance of the patient severe reactions being avoided or controlled by other drugs when they occur then progressive improvement is noticed in nearly every case

The size of dose required to produce the first reaction varies with the type of case and the degree of vascularity of the lesions In skin cases (B or B_3) with granulomata containing large numbers of acid fast bacilli small doses such as 3 to 10 grains produce reactions and doses less than 20 grains may have to be administered once a week for some months before the granulomata have become sufficiently absorbed for such doses to cease to cause reactions so *that it* is possible to administer larger amounts of potassium iodide In such cases once 30 *grains fail to* cause reaction it is generally possible to raise the dose to 60 straightaway and when with 60 grains the lesions fail to react to give 120 and then 240 grains with little delay

On the other hand in some skin cases with fibrous non vascular lesions no marked reaction may occur till 60 or 120 grains have been reached but the lesions

gradually become softened and more vascular. Then at a certain point marked reactions occur due to the iodide being able to penetrate the now more vascular granuloma. In such cases it is often necessary to reduce the dose as the flooding of the lesions with iodide causes fever and pain beyond the tolerance of the patient. Then as the lesions clear up the doses may be raised once more.

In cases with comparatively few bacilli, nerve cases (A_1) or early skin cases (B_1), the doses may be raised much more quickly. Iodide may be given daily rising from 5 grains by daily five grain increments till 30 grains is reached or till a reaction results, whichever happens first. Thereafter iodide is administered once a week. It is often possible with such cases to reach the maximum dose (240 grains) within six or eight weeks. In some however painful nerve reactions cause delay, i.e. in cases in which the nerve trunks are markedly affected.

In some cases there is no reaction until several doses of 240 grains have been given and then fever occurs with the swelling up of some gland or skin or subcutaneous lesion which had not been suspected of harbouring bacilli. In others there may be a reaction caused by a comparatively small dose, say fifteen grains, then when the fever and swelling have subsided very much larger doses are tolerated and no further reaction occurs, or there is none until the largest doses up to 240 grains have been reached. Certain lesions in the skin may persist in spite of repeated maximum doses, but counter irritation in the form of painting the skin with a one in three solution of trichloracetic acid in distilled water appears to throw the door open to the iodide and under this combined treatment resolution is accelerated.

The writer has not yet had time to determine the length of treatment required, but provisionally a rule has been laid down that, after all reactions have ceased, maximum doses must be taken for three periods of one month each with a month's rest after each period. Whether this will be sufficient entirely to sterilize the patient remains yet to be seen.

In skin cases which at the beginning of treatment show marked reactions not only is there the direct action of potassium iodide in breaking down the mechanism which protects lepra bacilli from the tissues, but there is also an indirect effect which helps to bring about their destruction. The breaking down of the leproma sets free antigens and these again cause an anti leprosy immunity and this immunity operates in causing further curative effect. Indeed the iodide treatment is not only a form of chemotherapy but also results in the production of effective auto vaccination. The less the infection however at the beginning of treatment the less the degree of immunity that it is possible to produce in this way. Conversely, we may hope to arrive at a very complete degree of sterilization in patients whose dosage has gradually raised with reactions at every step from small doses to maximum ones, the immunity in such cases being very considerable.

It will, however, require some years before the final effects of iodides in this direction can be determined.

The Administration of Potassium Iodide.—In the smaller doses this drug is be administered as a single dose at bed time dissolved in a large glass of water. Whe more than 60 grains are given the dose may be divided in two, half being taken a mid day and half at night.

One of the remarkable things about potassium iodide is its complete absence o toxicity, even in the maximum dose of 240 grains. The smaller doses frequentl cause catarrhal symptoms and even an iodide rash, but there is seldom any troubl with such symptoms in the larger doses. It is important to take plenty of wate both as a solvent for the iodide and afterwards and milk and ghee are said t diminish the symptoms of iodism when they are present. When a sever iodide rash occurs it is generally sufficient to omit the drug for 7 or 10 day till the rash diminishes and then the iodide may be continued, giving larger dose than previously if too strong a leprous reaction is not to b apprehended.

It is important that the bowels be well regulated—otherwise iodide may caus diarrhœa. It has generally been found that this diarrhœa is the result of th administration of iodide to patients who are suffering from constipation, chroni dysentery or other gastro intestinal disorders. We have seldom failed by simpl remedies to remove such disorders so that the patient has been able to take maximum doses without further trouble.

Iodides can be administered in large doses in most intercurrent diseases but in pulmonary tuberculosis special care must be observed. If the temperature is taken regularly it will be noticed in such cases that there is a febrile rise out of proportion to the other signs of leprous reaction and that the patient complains of cough. Iodide should be stopped at once, the sputum examined for acid fast bacilli and the physical signs in the chest carefully tested.

We have never noticed the appearance of albumen in the urine after even the largest doses, although over 95 per cent of the drug is excreted in the urine. In one case of diabetes the glycosuria had entirely disappeared by the time that 240 grains was reached and the general health of the patient had improved. When there are repeated small reactions, continuous administration of iodide twice a week may cause a certain amount of general weakness. Iodide may be stopped for a week and an iron and arsenic tonic given. But it is advisable that treatment be as continuous as possible consistent with the general health of the patient.

Iodide treatment may be given by itself or it may be combined with the *intra* venous injection of sodium hydnocarpate or the subcutaneous or intramuscular injection of hydnocarpus oil or esters.

When syphilis is present along with leprosy, avenyl (Hg 33) solution in hydno carpus oil may be injected twice a week for 15 injections while iodide is given *orally*. We have found this combined treatment very effective, the iodide and hydnocarpus oil benefiting the leprosy and the iodide and avenyl being effective in the treatment of syphilis.

II The Sedimentation of Erythrocytes as a Guide in the Use of Iodides in Leprosy

Pribram and Klein(1) found that the speed of sedimentation of erythrocytes was increased by the following conditions fevers malignant growths, decrease of total albumin content or of the number of erythrocytes in the blood increased viscosity cholesterin content and content of albumen end products while it was retarded in increased albumin content polycythæmia hypercholesterolæmia and cyanosis It is also well known that any condition which causes an excess of bile in the blood retards sedimentation very markedly

Drevfuss and Hecht(2) and others have shown that though the sedimentation test is useless in the diagnosis of tuberculosis it is more useful in the diagnosis of the activity of tuberculosis than the observation of the temperature chart

Puxeddu(3) showed that the sedimentation of the blood of lepers was accelerated and was still more rapid when leprosy was complicated with malaria He showed that this acceleration was due to charges in the serum of the patients and not in the red cells The opinion of all who have worked on this subject is that the velocity of sedimentation is increased in leprosy—much in nodular or skin cases less in mixed cases and least in nerve cases but no attempt has been made to use the sedimentation test as a guide to treatment

I hope to show in this paper that the blood sedimentation test is valuable in diagnosing cases in testing the reality of cures and in regulating treatment in leprosy, the test being used to ascertain the changes in the blood brought about by the administration of iodides

Various theories have been put forward regarding the significance of accelerated sedimentation but there seems to be agreement that it indicates the breaking down of tissues in the body That being so such acceleration may be expected and is found in many and various diseases especially when a drug like potassium iodide is used but in no other disease have we found the same marked and rapid acceleration produced by this drug which we find when iodides are administered even in small doses in leprosy

Our method of applying the test differs from that usually adopted in certain respects Sodium citrate (0 3 c c of a 5 per cent solution in saline) is drawn into an all glass 2 c c syringe 1 2 c cs of blood is then drawn from the patient's vein into the same syringe and mixed with the citrate solution in the barrel of the syringe by making a bubble of air to pass up and down and the mixture is then evacuated into a clean test tube Sedimentation is carried out in 1 c c pipettes graduated in 1 100ths The blood citrate solution is drawn up from the test tube into the pipette suction being applied by attaching a syringe by rubber tubing and pulling on the piston The pipette is then placed in a rack with the point downwards and inserted in a small hole bored in a rubber cork which prevents the contents escaping a rack holding 24 such pipettes is found convenient The level of the red cells is read off in 1 100th of a c c after 1½ hour and again after 2½ hours and an average taken of

these two readings This method has been adopted because of its delicacy, extrem simplicity and the short time required when large numbers of bloods have to b tested As has been described by other workers we found speaking generally that sedimentation increased in rapidity in proportion to the grossness of the lesions i e in proportion to the amount of leprous tissue and the number of bacilli in th body But this rule did not hold true in detail, as the sedimentation wa accelerated whenever a reaction took place and retarded when the reactior passed off

Signs of Reaction —What we term ' the reaction ' in leprosy is a well known phenomenon though its significance has often been misunderstood Its signs and nature have been dealt with in another paper and it is sufficient to mention here that it is accompanied by a rise of temperature swelling up and vascular engorgement of existing lesions and the appearance of fresh rose coloured nodules in the skin which disappear again in a few days These phenomena are the result of the iodide causing the breaking up of leproma

Sedimentation as a Test in Early Leprosy —While a normal blood shows a sedimentation index of 16 to 20 the blood in leprosy often shows acceleration to 50 60 or even 70 in the third stage, i e , in cases in which there is a large amount of leprous granuloma present In early cases on the other hand, such low figures as 10 or 15 may be obtained The sedimentation test is in itself of no value in making a diagnosis of leprosy , but in doubtful cases sudden acceleration of sedimentation following a large dose of potassium iodide is a very strong indication that leprosy is present This test is very delicate and it may give positive results even when the ordinary clinical signs of reaction (rise of temperature and swelling up of lesions) are absent This test is also of use in leprosy contacts who are otherwise in good health and have a low sedimentation index There may be acceleration from 15 or 18 to 30 or 40 within 24 or 48 hours of the administration of potassium iodide If this is accompanied by the swelling up of glands or if doubtful patches become more prominent or suspicious patches are noticed where none were evident before the diagnosis in favour of leprosy is strengthened It must however be remembered that latent streptococcal staphylococcal and other infections may also be lit up by potassium iodide and cause acceleration of sedimentation and that therefore a positive result with this test, especially if it is not very marked is of itself not absolutely diagnostic of leprosy

Sedimentation as a Test of Elimination of Leprosy from Body —As has been stated above the sedimentation of erythrocytes is accelerated in leprosy and the acceleration is in proportion to the amount of leprous granuloma present in the body When potassium iodide is administered in small doses *gradually rising to larger* ones and sedimentation is tested frequently say once or twice a week, it is found *that at a certain point increased acceleration occurs* This may or may not be accompanied by other signs of reaction If iodide is discontinued the sedimentation is retarded again to approximately the previous rate Thus in a case in the third stage (B, or B_3) the sedimentation may be 30 or 40 On administering a suitable

dose (say 5 to 20 grains) of iodide, it will accelerate to 60 or 70 and then fall to the previous rate As treatment proceeds and leproma is eliminated the dose required to accelerate to 60 or 70 becomes greater, and if iodide is discontinued for some days it may be noticed that the index level is gradually falling At last a point is reached at which even a massive dose of iodide like 240 grains fails to cause acceleration, but if this amount is continued twice a week a subsequent dose may cause marked acceleration once more due to repeated doses having at last succeeded in penetrating and destroying some of the remaining less penetrable lepromata In the end even massive doses fail to produce any result and the presumption is either that no more leproma is left or that it is in a form or in a part of the body which iodide cannot reach and break it down It will be found, however, that even at this stage the sedimentation index may remain high and only fall to normal very gradually

The Use of the Sedimentation Test in Regulating Treatment—Although this test is not absolutely essential for the regulation of potassium iodide treatment in leprosy, much help in this direction may be derived from its use A persistently high sedimentation indicates the grossness of the infection and general low resistance of the patient, while, in the absence of clinical signs, either focal or general, a rapid increase of the rate of fall of the blood cells shows that a breaking down of leproma is taking place in the internal organs In both cases there is an indication for caution in increasing the size of the dose When, however, in spite of a sharp rise of temperature following the administration of iodide, there is no marked acceleration of sedimentation, treatment may be pressed with more confidence

REFERENCES

(1) PRIBRAM, H, and KLEIN, O (1923) *Acta Med Scandin* 58, 109—312 May 22nd p 132
(2) DREYFUSS W, and HECHT, P (1922) *Munch Med Woch*, 69 No 21, May 26th, p 775
(3) PUXEDDU, E (1924) *Riforma Med.*, Vol XL, No 22 June 2nd, pp 507—509

THE REACTION IN LEPROSY AND ITS CONTROL

BY

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Nature of Reaction —What we have referred to as the *reaction* in leprosy is perhaps the most striking and important and at the same time the most misunderstood phenomenon connected with that disease The signs of reaction are as follows :—

(a) The swelling up of lesions When the lesions are in the skin this clinical appearance forces itself upon the attention of the patient and his friends, especially if the lesions are on a conspicuous part of the body The swelling is accompanied by marked erythema which shows up best in light-coloured skins When the lesion is in a nerve trunk the latter becomes thickened often to over 5 times its original diameter The pain may be very intense and the functions of the nerve are to a large extent held in abeyance resulting in anæsthesia, paresis and various trophic changes in the parts supplied In some cases there is marked swelling up of such organs as the liver and spleen, the testicles and lymph nodes

(b) Another phenomenon is the appearance of fresh, rose coloured nodules in many cases due to bacilli being set free from reacting lesions and carried in the blood stream to the skin or subcutaneous tissue of other parts of the body where they form emboli in the capillaries This results in the formation of nodules which disappear again in a few days

(c) A third sign of reaction is a rise of temperature, generally, but not always in proportion to the other symptoms

(d) Other accompaniments of reaction which may be detected in the laboratory are accelerated sedimentation of erythrocytes bacillæmia and leucocytosis

(2) *Causes of Reaction* —It is impossible as yet to be sure how a reaction is caused, but the following hypothesis appears to give the most likely explanation Potassium iodide is well known to have an affinity for injured and dying cells There are indications that it acts in leprosy by destroying and breaking up lepra cells which have been invaded by, or have ingested bacilli and in which the bacilli have multiplied gradually causing destruction of the nucleus and cytoplasm These cells are at different stages of ripeness and smaller doses of iodide will cause bursting of the riper cells while larger doses are necessary to destroy the less ripe cells Cells

which although they contain a few bacilli, have not begun to be damaged are probably unaffected by iodide however large the dose The breaking down of the cells leads to the bacilli being brought into contact with the tissues from which they had been isolated and protected by the cytoplasm of the containing cells and this leads to leucocytosis and the ingestion and destruction of the bacilli

There are other causes of the breaking down of this protective mechanism, such as the lowering of the general resistance of the patient, fevers, various bowel disorders and certain drugs and irritants when injected There is, however, no cause more certain and powerful in inducing reactions than the iodides, and the use of potassium iodide in the treatment of leprosy has given us a unique opportunity of studying these phenomena

(3) *The Duration and Severity of Reactions*—While there may be many circumstances determining the length and severity of a reaction the following two facts are the most important —

(*a*) The state of the patient's general health The low general resistance of the patient may be sufficient of itself to cause a prolonged reaction which may continue until the general health is improved Or again, the general condition though poor may not be sufficiently low to induce reaction but a slight superadded cause may be sufficient to produce and prolong a reaction, which it could not have caused had the general resistance been greater likewise chronic bowel disorders may either create reactions or lead to such a state in the body that comparatively slight causes produce them

(*b*) The duration and severity may depend upon the type of case, upon the amount of leprous granuloma in the body upon the amount of vascularity and penetrability of the lesions and upon the ripeness of the cells When potassium iodide is first administered in third stage skin cases, even minute doses will often produce severe and prolonged reactions This is doubtless due to innumerable patches of highly vascular leproma in the skin lymphatic tissues and internal organs which are easily penetrated by the iodide carried by the blood and thus the protective mechanism of the contained bacilli is broken down But later when such lesions have been cleared up and only the harder more fibrous and less vascular granulomas and less ripe lepra cells are left much larger doses are necessary to produce any reaction at all

(4) *The Importance of Reactions in the Treatment of Leprosy*—Many workers have regarded the reaction in leprosy as a condition to be dreaded and avoided The patient certainly appears to be worse Comparatively innocent looking or quite unnoticed lesions suddenly become red swollen and angry, and fear of this is quite natural as long as the true condition is not understood When reaction takes place in nerves the pain may be severe and this is dreaded by patients Then there is the belief which most leprologists have held that if bacilli are set free in the blood stream as undoubtedly occurs during reactions the disease will spread and new lesions will be formed But though bacilli are carried to new skin areas and form emboli as shown by the appearance of the rose coloured nodules, the writer's

experience shows that fresh lesions are not formed the rose coloured nodules disappear again in a few days and leave no trace This statement, however, only applies if —

(a) The case is in the 3rd stage (B_3) and there is abundant granuloma to break down in the 2nd stage a *single* reaction often is followed by a marked increase of the disease

(b) The reaction is caused by potassium iodide and the administration of this drug is persisted in Potassium iodide seems to have the power not only to set free bacilli but also to follow them up and prevent their settling down to form new lesions In the administration of potassium iodide, therefore it is very important that the treatment should be progressive and continuous, progressive in order that excessive reactions beyond the tolerance of the patient may not be produced and continuous in order that especially in 2nd stage cases fresh lesions may not be formed

If it be proved that the breaking down of the leproma and the bacillæmia resulting do not cause an extension of the disease it stands to reason that this breaking down must be the most beneficial thing possible in the treatment of the patient provided that it is accompanied by adequate elimination of the broken down material The elimination power varies in different patients and in the same patient at different times and iodide must be administered with this fact borne in mind, the dose of iodide regulated accordingly and everything possible done to increase elimination

Regulating the Reaction —One of the great advantages of iodide over other forms of leprosy treatment is the ease with which reactions are regulated This is due to three factors

(1) Its rapid absorption and elimination from the body Large doses are said to be entirely absorbed from the gastro intestinal tract within half an hour and they are almost entirely eliminated chiefly through the kidneys within 48 hours

(2) Even small doses (1 grain or less) will cause reaction in gross infections and therefore there is a very large range of increase possible

(3) Potassium iodide is given orally and even in large doses is practically non toxic In fact the larger doses can often be taken with less inconvenience than the smaller ones

(4) Although at first prolonged reactions may be induced by small doses in third stage (B_3) cases the patient soon reaches a point at which reactions occur only when there is a high concentration of iodide in the blood and pass off as the drug is eliminated from the body

It is therefore chiefly at the beginning of the treatment of skin cases that it is necessary to adopt means to limit the intensity and duration of reactions We have noticed that several of the heavy metals such as copper and mercury, have this power when given in small doses but we have found that antimony (0 02 gramme doses of pot antim tart) given intravenously every 2nd day is the most useful If the reaction produced by iodide lasts for more than 3 days antimony should be given and continued till the temperature becomes normal

On the other hand if the reaction is in the nerve trunks, adrenaline has been found effective in checking it Three minims of P D & Co's 1 1000 solution of adrenaline chloride is mixed with 30 or 40 minims of normal saline and injected intramuscularly If the nerve pain is not relieved in 5 minutes, the dose should be repeated Whatever the therapeutic effects of iodides may finally be proved to be, it is difficult to imagine any remedy which will break down leprous lesions more rapidly In most cases delay in recovery is not due either to the toxicity of iodides or to lack of their power in destroying diseased areas, but to the fever and pain caused by the breaking down of lepromata being beyond the endurance of the patient, a limited amount of the drug having to be given for this reason

Reactions in the Internal Organs —Reactions are common in lymphatic glands in the groins and axillæ and also in the iliac and mesenteric glands In two cases, the first small doses produced jaundice, apparently due to reaction taking place in the liver or biliary passages In several others it was the testicles which reacted repeatedly and prevented the raising of the dose In one case there was marked enlargement of the liver and spleen necessitating a careful diagnosis from kala azar As a rule these highly vascular lesions are soon cleared up by small doses of iodide and it is then possible to raise the dose and create a higher concentration of iodide in the blood which is able to act on the less vascular and more fibrous lesions, or on the less ripe lepra cells

In them the effect of iodide is often a gradual one large doses of 120 or 240 grains producing little apparent effect at first but in the end causing softening of hard nodules, the contents of which may liquefy and break down Later when this softening process has gone beyond a certain point the dose may have to be reduced, as much smaller doses induce very marked reactions in the lesions which have now become vascularized In very hard lesions the use of such counter irritants as trichloracetic acid markedly hastens the action of iodides by increasing permeability

The writer's experience tends to show that when a lesion which has reacted markedly and repeatedly ceases to react in spite of larger and repeated doses of iodide, the cessation is due to the complete elimination of lepra bacilli from the lesion Further confirmation of this is, however still required

When marked improvement under iodide treatment is not obtained or when such improvement is at first induced but later is not maintained, it is well to combine with it hydnocarpus injections

DISCUSSION

Dr C Natesan Moodeliar (Madras) I am on the Leprosy Relief Committee constituted by the Government of Madras I have had opportunities of reading the pamphlets issued by Dr Muir I am glad that I see him in person to-day From time immemorial chaulmoogra oil has been considered to be a remedy for this disease by the Ayurvedic physicians of India especially of southern India So have been the preparations of arsenic and copper especially the latter Many stories are told of Sadhus

appearing and administering ¼th or ⅛th of a grain of a preparation of copper (*Tamra Vasmam*) and the disease disappearing miraculously I do not know how Dr Tampi, when he was enumerating the various preparations administered by Ayurvedic physicians, left out this important one As to chaulmoogra oil itself I have known of a case in the incipient stage cured by the inunction and oral administration of the drug Side by side with this the patient was also treated with arsenic and a little nux vomica with intervals according to the disposition of the patient for over two years I have known also of another case in which the disease was arrested by the same treatment As to the potassium iodide treatment it may be good as a preventive but as a curative, it is a troublesome treatment Between the minimum and maximum doses some patients exhibit idiosyncrasies with swelling of the tongue, watering of the eyes, blocking of the nose and aching of the head *The patients are thus frightened away from the* doctor It may be all right in a hospital, but it seems to be impossible for a private medical practitioner Dr Tampi only experimented with it in a hospital May I request Dr Tampi having the medical side of the whole of Travancore administration at his disposal to extend his researches towards chaulmoogra and the preparations of copper? May I also request Dr Muir to devote his attention in this direction? Dr Muir was telling us that treatment centres become very popular I ask Dr Muir whether it would not be advisable to have a hospital where the patients can be segregated (of course voluntary segregation) instead of treating them and sending them away Sent away thus they continue to be centres of infection, so that, if one batch is cured, another one will take its place Thus the disease will not be eradicated (eradication is our goal) but will continue to exist though not in such large numbers In the city of Madras when there was a Leper Asylum scarcely a leper was seen in the streets When the asylum was abolished and was removed to a place in a neighbouring district (Therumani in the Chingleput district) lepers were seen everywhere They congregate where the buses stop where the trams stop in front of bazaars and in front of poor feeding houses They are now a menace to the city May I ask Dr Row, whether the disease was completely cured by his treatment and did not reappear? If it was only a temporary subsidence one can get this result with injections of hydnocarpate of soda

Dr John M Henderson (Bengal) Certain points arise in connection with the papers contributed by previous speakers I wish first of all to congratulate Dr Row on his most excellent communication and I trust that his investigations will continue I seek information on two points only (*a*) how does he explain the action of his autolysate on cases showing trophic ulcers? (*b*) What criteria did he adopt to estimate '*cure*' in his nodular cases? Although clinical improvements may often be marked, deep foci may exist in the skin or deeper tissues and these may escape detection

With regard to Dr *Tampi's paper several points arise* (*a*) *It is interesting to note* that several of his cases give a previous history of snake bite rat bite or spider bite one frequently encounters patients who volunteer the statement that trauma took place at the site of a presently existing depigmented patch The workers at Culion (Philippine Islands) also found in a study of leper children that a depigmented patch frequently appeared on the site of a previous scabies lesion (*b*) Dr Tampi has referred to the question of filariasis and leprosy I have no experience of this on a large scale but I should like to draw attention to *the following findings —I excised*

about a dozen inguinal lymphatic glands for histological purposes and in one there was found a portion of an adult filarial worm The remainder were submitted to Col Acton, pathologist at the School of Tropical Medicine, and he was of opinion that several of them manifested histo-pathological changes indicative of recent filarial infection (c) With regard to the mortality of leprosy I think Dr Tampi ought to differentiate deaths from leprosy *per se* and deaths from complications arising in the course of the disease Pineda in Culion examined some 300 cases from this point of view and concluded that in only about 28 per cent were deaths directly attributable to leprosy itself The remainder were due to secondary complications and especially tuberculosis and nephritis (*l*) Dr Tampi thinks that depigmented patches are similar to some corresponding lesions found in peripheral neuritis With this view I cannot concur We have examined numerous depigmented patches and I am of opinion that the changes there are essentially similar to those found in more marked and definitely leprous lesions, though naturally less in degree

Dr R Roe (Bombay) It is gratifying to see Dr Muir showing signs of changing his venue in the treatment of leprosy From his fixed ideas on the value of chemotherapy in this disease by gynocardates morrhuates ethyl esters moogrol hydnocarpate E C C O etc, etc, he has at last veered round to the more rational vaccine therapy which he calls auto vaccine liberated under the influence of potassium iodide It was amusing to learn that other remedies in the shape of tuberculin and other microbic vaccines sterile milk and turpentine operated beneficially through the production of protein shock as though the specific action was reserved only for chaulmoogra oil ethyl ester etc As to potassium iodide acting as an indirect auto vaccine by liberating the *bacilli lepræ* one would require a fuller demonstration of this than the assumption we have heard to day Even under this assumption one has to face the risk of over mobilization of *B lepræ* and this beyond the power of control The charts shown on the screen appeared to my way of interpreting them more those of iodine tolerance curves or the phenomena of iodism than reaction in leprosy and I submit it is dangerous to accept generalizations in the absence of rigid control charts of the action of potassium iodide in apyrexial tubercle and other granulomatous inflammations

Apropos of specific vaccines it is a pity no mention was made of Dr Hussain's vaccine which gave such striking results in the case reported early this year by Dr Graham Little in the *British Medical Journal* An ideal vaccine would be one made from leprosy bacilli themselves but in the absence of cultures of *B lepræ* the next best thing is to fall back on an allied antigen and depend on group antibody formation That is why and how the autolysate made from tubercle bacilli came into being and I hope it will commend itself to the acceptance of this Association as a curative agency

Dr C D Fsch (Central Provinces) When can we call a case cured? We who follow out the treatment of leprosy with hydnocarpus oil and creosote etc, all get very gratifying results but when can we call a case cured? I am informed that many cases treated in the Philippine Islands who have been discharged as cured having been kept under close observation for a period of 2 or more years when they return to their former way of living frequently develop a more serious form of leprosy than they had in the beginning

Dr Isabel Kerr (Hyderabad Deccan, B India) Among the 300 leprous patients *undergoing treatment at Dichpalli, Nizam's Dominions, the following percentages show* the results of treatment by hydnocarpus esters —

17 per cent, symptom free
45 per cent being much improved
35 per cent, improved
3 per cent, worse or dead

Of the infective cases treated, 63 per cent became non infective, many of the remaining ones becoming so after the results of treatment were made

It is a mistake to think that any one who goes out to treat leprosy with a hypodermic syringe in one hand and a bottle of hydnocarpus oil *in the other is going to get success* with his cases One has to bring every possible factor in the situation to one s aid All possible complications have to be got rid of and every possible help utilized Resistance natural resistance has to be developed To this end leprous patients must have *healthy* surroundings *At Dichpalli* we are fortunate in having our hospital erected on high ground in a dry climate The patients have regular exercise in order to re develop the flaccid muscles and general torpid physical condition We are also particular to keep the skins of our patients as healthy as possible In fact we seek to utilize every possible help in order to attain our end

Dr R B Tandan (Jodhpur, B India) I want to tell you something about the efficacy of Amla in Leprosy (आवला)

In 1906 a Calcutta merchant, aged 35 came to me for treatment with one thumb, two fingers, one great toe and 2 toes, one side of face and lips swollen up and red I could not promise him cure He left I saw him 2 months after in the market very much improved, with the redness and œdema almost gone On inquiry he said that he took dry 'amlas' 2 chhataks and in this he poured the juice of green 'amlas' in a shallow china clay enamelled vessel and dried it in the shade He poured the juice again on it as soon as it was a little dry He did the same 20 times In Ayurveda they advise this to be done 40 times 2 *chhataks of this preparation taken during the* course of 7 days morning and evening produces wonderful effects I repeated this treatment in 1910 on a mate in charge of jute coolies, a Hindu Rajput, a robust man, 22 years old He had a big non æsthetic area on his thigh, buttock and leg It did him

When he came
ent was repeated
she was much

benefited With regard to the 1st case I see him every year He takes the same medicine every August and March He takes no salt during the course and confines himself to his room To a lay man he does not look a leprosy case Only a medical man could find out by very close attention that he had got latent leprosy

In Sujangarh, a town of 12 000 people in Bikaner State *there were 6 lepers* The place has a municipality and proper conservancy arrangements The water inside the town is nowhere more than 9 feet deep poisonous and blackish Ludnun is 6 miles *towards* the south west with a population of 10 000 people on a hill, having only from 2 to 9 feet of sand over the stony underground Water is above 100 yards deep There

are 13 lepers there Why this difference ? Simply because there are no conservancy arrangements at all at the latter place

Dr B Saha (Bengal) We are far from the goal of a cure of leprosy

The numerous drugs used one after another refute the contention that we have got a specific Bacterial diseases as opposed to protozoa, broadly speaking, have not yet got a specific cure except diphtheria and tetanus antitoxin and staphylococcus vaccine in multiple boils Chronic diseases, having a long course with long periods of remission must be carefully considered before one ascribes credit to the treatment Clinical cures are the only criteria of cures in spite of our serology bacteriology and biochemistry

Col. I Froilano de Mello (Portuguese India) Is of opinion that treatment of leprosy nowadays comprises means which can be divided into four classes Physiotherapy, phytotherapy metallotherapy and vaccine therapy Having experience only on phytotherapy and metallotherapy he agrees with the results obtained by Dr Muir and the Philippine authors with chaulmoogra derivatives He informs the Congress of good results of karpothrocate of sodium and other derivatives from the Brazilian plant *Karpothrocle brasiliensis*

Antimony has had, in his hands, only the result of healing ulcers no other improvement We cannot actually say that one patient is cured and it is for this that the term 'paroled' of Philippine authors is a happy one

Another point of scientific importance We have no scientific basis to consider broken bacilli as degenerate forms of the leprosy bacillus which may act as a bacterioscopical test of amelioration Even in tuberculosis, where such opinion prevailed four years ago the question was adjourned for further discussion and the *Koch B* is culturable

If you examine a patient before and after treatment with evident ameliorations you find a total reduction in all the forms of bacilli and in no way a change into the relation of homogenous to broken forms $\frac{H}{Br\ B}$ which would be the case if such transformation should occur

Dr R H H Goheen (Bombay) Gave a history of the drugs he had used in his leprosarium In his experience improvement had not been maintained

Dr E Muir (Bengal) Emphasized the point that although we had not got as yet a 'specific' for leprosy yet we had remedies which are capable of removing all active signs in a large majority of cases, and that many patients who were treated several years ago still remain symptom free The importance of propaganda treatment survey centres was mentioned as by these means early cases were reached and the infection was cut off from the coming generation by rendering advanced cases non infectious by treatment By these centres also interesting data were forthcoming as to the reason why leprosy was common in certain areas

RECHERCHES SUR LE SANG DES LÉPREUX

PAR

Major V. G. F. LABERNADIE

ET

Z. ANDRÉ

Pondichery, Etablissements français dans l'Inde

La difficulté du diagnostic de la lèpre au début et parfois au cours de la maladie, les résultats des *méthodes sérologiques employées dans la syphilis et la tuberculose* ont depuis longtemps orienté les recherches des léprologues vers des procédés de laboratoire susceptibles d'étayer un diagnostic hésitant.

Bordet-Wassermann

La valeur de la réaction de Bordet-Wassermann dans le diagnostic de la lèpre est encore en discussion, mais il semble de jour en jour qu'à mesure que cette réaction est exécutée avec plus de précautions, elle est trouvée chez les lépreux plus souvent négative qu'autrefois, ainsi qu'il ressort des travaux de Mathis(1), Van den Branden(2), Pais(3), etc.

Nous avons recherché la fixation du complément en présence de l'antigène syphilitique par le procédé de Mutermilch (dérivé du Hecht-Bauer). Les réactions furent exécutées avec le plus grand soin et après recherche précise de l'index hémolytique. Voici les résultats obtenus dans ces conditions sur 48 sérums provenant de la léproserie:

4 formes maculeuses relativement récentes: 3 résultats négatifs, 1 positif faible.
4 " tégumentaires: 3 résultats négatifs, 1 positif fort.
7 " tubéreuses: 1 résultat négatif, 6 positifs forts.
9 " mixtes: 4 résultats négatifs, 5 positifs faibles.
16 " nerveuses: 10 résultats négatifs, 6 positifs forts.
8 " nerveuses mutilantes: 4 résultats négatifs, 3 positifs faibles, 1 positif fort.

Sur ces 48 lépreux nous avons donc obtenu 25 résultats négatifs, soit 52 pour cent.

Si l'on considère que sur environ 700 réactions que nous avons systématiquement appliquées au sérum de tous les entrants à l'hôpital et de la plupart de nos consultants, nous avons rencontré une moyenne d'environ 50 pour cent de résultats

positifs, il faut convenir que la réaction de fixation appliquée à Pondichéry au sérum des lépreux n'a aucune signification Il est permis à ces malades aussi bien qu'aux autres d'être syphilitiques dans la proportion de 1 sur 2

* * * *

Nous ne citerons que pour mémoire la réaction de Gaté Papacostas (formol gélification) et le test des globulines de Ray (floculation des sérums en présence d'eau distillée) Froilano de Mello et Barreto(1) ayant montré qu'elles sont sans valeur pratique aussi bien pour le diagnostic de la lèpre que pour celui de diverses autres maladies

* * * * *

REACTION DE MATEFY

Une autre méthode de floculation la réaction de Matefy(6) a récemment attiré l'attention Cette réaction d'abord appliquée à la tuberculose et qui s'est avérée sans valeur dans le diagnostic de cette maladie consiste à ajouter 0 c c 2 de sérum à 1 c c de solution récemment préparée Les sérums sains ne floculeraient pas les sérums lépreux(6) floculeraient entre 0 et 75 minutes (au delà la réaction n'est pas valable) Marras(5) l'a trouvée constamment positive chez les lépreux examinés(20) et les tuberculeux pulmonaires négatives dans les autres localizations tuberculeuses et diverses maladies (syphilis dermatoses)

Nous avons appliqué cette réaction à 50 sérums de lépreux avérés (internés à la léproserie) et à 26 sérums de malades divers non lépreux et voici les résultats obtenus (voir tableau annexe)

(1°) Sept sérums seulement sur 76 n'ont pas floculé 5 sur 50 lépreux 2 sur 26 non lépreux

(2°) Le degré de floculation n'est guère plus caractéristique chez les non lépreux =12 floculations faibles 2 moyennes 10 intenses chez les lépreux — 12 floculations faibles 11 moyennes 2? intenses

(3°) Comparés aux formes cliniques de la lèpre les résultats ne sont pas très significatifs

Dans 4 formes maculeuses relativement récentes 1 floculation moyenne 3 intenses
5 tégumentaires 1 floculation faible 2 moyennes 2 intenses
, 7 tubéreuses 1 floculation nulle 2 faibles 2 moyennes 2 intenses
10 mixtes 1 floculation nulle 6 faibles 2 moyennes 1 intense
16 nerveuses 3 floculations nulles 1 faible 2 moyennes 10 intenses
8 nerveuses mutilantes 2 floculations faibles 2 moyennes 4 intenses

C'est dans les formes nerveuses qu'on rencontre le plus grand nombre de floculations intenses mais aussi de floculations nulles

Contrairement aux résultats obtenus par Marras cette réaction ne nous a paru être d'aucun secours pour le diagnostic sérologique de la lèpre *au moins dans sa forme* actuelle

Sédimentation Globulaire.

D'apres Siwinski(20), c'est Biernacki le premier qui en 1894 97 attira l'attention sur l'intérêt diagnostique de la vitesse de sedimentation des hematies dans les états pathologiques Mais il faut attendre une vingtaine d'années et arriver à Fahreus(7, 8) et surtout à Westergreen(9, 11) et à Linzenmaier(10, 13) pour que des techniques d'execution facile soient publiées et bientôt essayées per beaucoup d'experimentateurs qui leur imposeront de nombreuses modifications de detail(20).

Mais le principe reste le même : une faible quantité de sang total rendu incoagulable par le citrate de soude est placée dans un tube de faible diamètre. Peu à peu, les globules vont se deposer au fond du tube laissant le plasma surnager La vitesse de sédimentation s'exprime soit par le temps que met le niveau supérieur des globules à atteindre un trait marqué d'avance (Linzenmaier et derivés) soit par l'espace parcouru en un temps donné par ce même niveau globulaire (Westergreen et derivés)

D'apres les recherches de divers auteurs l'accélération de la sédimentation donnerait des indications intéressantes en gynécologie(7, 8, 12) ainsi que pour le diagnostic et surtout le pronostic de la tuberculose(11, 12, 14, à 18) enfin dans certaines maladies mentales(20) Gilbert, Tzanck et Cabanis(19) ont, au Congrès de Dermatologie de Bruxelles en 1926 montré que la vitesse de sédimentation est augmentée chez les lépreux, et que ses variations permettent de suivre l'évolution de la maladie et de controler la thérapeutique instituée. Cette communication nous a incité a faire quelques recherches sur nos lépreux de Pondichéry

Technique employee—Parmi les variantes de Westergreen, nous dirons que notre technique est à peu pres celle de Cordier et Chaix(14) ou de Kosticht(18). La solution anticoagulante employee est du citrate de soude à 3 gr 8 pour 100 gr d'eau distillée

Il nous a paru très difficile pendant la ponction veineuse ' d'agiter en tous sens ' la seringue renfermant la solution anticoagulante pour assurer l'homogeneité du mélange et éviter les coagulations partielles qui risquent de fausser les résultats Nous employons tout simplement un tube à essai ordinaire (14 mm diam) ou un trait bleu marque les 5 c cs mesurés à la pipette avant séchage et stérilization Dans ce tube a repere sterilisé, on introduit immédiatement avant la ponction veineuse, 0 c c 5 de solution citratée stérilisée La ponction est faite sans seringue avec une aiguille nue et le sang s'écoule dans le tube tenu par un aide accroupi qui agite le mélange, verifie l'affleurement du sang au trait bleu et dès qu'il est réalisé, sépare le tube

Ces 5 c cs ainsi bien mesurés continuent à être agités et sont versés dans un tube à hemolyse du modele courant L'heure est notée ainsi que la hauteur totale du sang (H) qui mesure de 55 à 65 mms La sédimentation commence prèsque immédiatement. Au bout d'une heure, on mesure la hauteur du sediment (h) en partant du fond du tube. La différence (H-h) donne la hauteur du plasma,

c'est à-dire le chemin parcouru par la couche supérieure des globules en une heure de temps

Pour rendre les résultats plus comparables on établit le pourcentage de la vitesse de sédimentation :

si pour H on a $H-h$

pour 1 on aura $\frac{H-h}{H}$

pour 100 on aura $\frac{(H-h)\times 100}{H}$

On admet que les chiffres trouvés chez les femmes sont parfois plus élevés que chez les hommes

L'expression " pour cent " ne doit pas tromper Il s'agit là d'une commune mesure et non d'un maximum réalisable Pour aussi rapide, aussi complete que soit une sédimentation il n'en reste pas moins le volume minimum de la masse globulaire qui ne peut s'annuler ni même se réduire a notre avis à moins de 25 pour cent de la hauteur totale La vitesse de sédimentation maxima ne nous paraît donc pas pouvoir dépasser 75 pour cent, chiffre que nous n'avons d'ailleurs jamais observé

* * * *

Nous donnons plus loin la liste C de vingt témoins, non lépreux et également indemnes de tuberculose, d'affections fébriles, de psychoses, puisque ces maladies accélérent la sédimentation globulaire Les chiffres obtenus vont de 31 à 50 pour cent, sauf deux chiffres extrêmes 11 pour cent, et, chez une femme, 53 pour cent, ils donnent comme moyenne générale 41 pour cent

Nous considérons que chez les individus indemnes des affections ci dessus, l'espace parcouru par les globules en une heure est inferieure à 50 pour cent de la hauteur du sang total

* * * *

Dans la liste B, nous avons inscrit à la 3è colonne les chiffres de sédimentation obtenus chez des lépreux ne présentant pas de signes bacteriologiques ou stéthoscopiques de tuberculose, indemnes aussi d'affections fébriles et de psychoses Sur 41 lépreux les chiffres ne sont que 8 fois égaux ou inférieurs à 50 pour cent, ils sont 33 fois compris entre 51 et 71 pour cent et donnent comme moyenne générale 58 pour cent Par rapport aux formes cliniques

6 formes maculeuses ou tégumentaires (macules et quelques tubercules) donnent une sedimentation moyenne de	53 pour cent
7 formes tubéreuses (quelques macules surtout des tubercules) .	66 „
7 „ mixtes (formes précédentes enrichies de lésions nerveuses)	60 „
21 „ nerveuses (retrocession plus ou moins complete des symptomes cutanés) ..	56 „

Il est intéressant de remarquer que la vitesse de sédimentation semble augmenter avec la gravité des symptomes tégumentaire qui sont à la base des formes les plus évolutives, et diminuer avec l'apparition des grands symptomes *nerveux et leur* systématisation plus ou moins exclusive, qui correspond à la demi guérison spontanée, à la 'cristallization' décrite par les classiques

Nous avons aussi entrepris des recherches, *encore en cours, sur l'action du* traitement anti lepreux sur la sédimentation Comme l'ont expose Gilbert et ses collaborateurs(19) elle nous parait nettement influencée par les derivés du chaulmoogra

* * * *

Conclusions

(1°) *Le Bordet Wassermann* (Hecht-Bauer Mutermilch) n'est pas chez les lépreux plus souvent positif que dans l'ensemble de la clientèle hospitalière de Pondichéry

(2°) *La r action de Matefy*, au moins dans sa forme actuelle, ne donne aucun renseignement pratiquement valable pour le diagnostic de la lepre

(3°) *La sédimentation globulaire* est en général nettement accelerée chez les lépreux particulièrement dans les formes tuberculeuses* et mixtes En presence d'un cas suspect de lepre, chez un sujet indemne de tuberculose, d'affections fébriles, de psychoses, *elle peut donner d'importantes indications*

Index Bibliographique

(1) MATHIS (1923) — IIIe Congrès international de la lèpre—Strasbourg
(2) VAN DEN BRANDEN (1926) — *Ann Soc Belge Med Trop*, Tome V, No 2
(3) PAIS (1927) — *Giorn Ital di Dermat e Sifil*, Tome LXVIII
(4) FROILANO DE MELLO et BARRETO (1926) — *Bull Soc Path Exot* p 127
(5) MARRAS (1926) — *Rev Sud Amer* Tome IX, Déc, p 1132
(6) MATEFY (1923) — *Deut Med Klin*, No 21
(7) FAHREUS (1917) — Congrès de Chirurgie et Gynécologie Stockholm Déc
(8) *Idem (1918)* — *Bioch Zeits*
(9) WESTERGREEN (1919) — *Acta Medica Scandin*
(10) LINZENMAIER (1920) — *Arch fur Gyn*
(11) WESTERGREEN (1921) — *Brit Jour Tuberc*, Avril
(12) GULISSAZ (1923) — *Rev Méd Suisse Romande* Mai
(13) LINZENMAIER (1923) — *Munch Med Woch*, No 40
(14) CORDIER et CHAIX (1924) — *Lyon Médical* Sept
(15) POPPER et KREINDLER (1924) — *Soc Med Hop Bucarest*, Juin
(16) *Idem* (1924) — *Presse Médicale* Déc
(17) SALOMON et VALTIS (1925) — *Presse Médicale*, Mai
(18) KOSTITCH (1925 26) — *Thèse Lyon*
(19) GILBERT, TZANCK et CABANIS (1926) — Congrès Dermatologie Bruxelles Juillet
(20) SIWINSKI (1926) — *Presse Médicale*, Sept

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* Après la rédaction de cet article, nous avons eu connaissance du travail de Landeiro sur le meme sujet publie par la Société Portugaise de Biologie en *1926 (cf Bull Institut Pasteur, 1927,* p 154) Nous sommes heureux d'etre arrivés aux memes conclusions que lui

TABLEAU A.

Réaction de Matefy chez des non lépreux

Numéros	Age et Sexe.	Atteint de	Matefy.
1	63 H.	Rhumatisme	+++
2	27 F	Douleurs articulaires	+++
3	65 H	Chancre	+++
4	40 H	Bronchite	+
5	23 H.	Blennorrhagie	+
6	27 H	Dyspepsie	+++
7	40 F.	Métrorrhagie, Diarrhée	+++
8	35 F	Métrorrhagie	+
9	20 F	2e Vaginite	+
10	25 F	Grippe	++
11	46 H	Bronchite	+
12	40 H	Tuberculeux agonisant, mort, 1 heure après	0
13	63 F.	Fatigue générale	+++
14	50 F.	Ascite	0
15	25 F	Céphalalgie	+++
16	23 H	Bronchite	+++
17	32 H	Chancre	+
18	38 H	Tuberculose pulmonaire	+
19	25 H	Tuberculose pulmonaire	+
20	3 H	Convulsions	+
21	29 F	Mère de cet enfant	+
22	28 F.	Métrite	+
23	23 F	Métrite	+
24	45 H	Rétrécissement uréthral	++
25	18 H	Chancre	+++
26	34 H.	Ulcères	+++

A/s Matefy :— + signifie floculation faible ; ++ = floculation moyenne ou forte +++ = floculation intense. Les floculations se sont toujours produites dans les premières minutes ou pas du tout.

Tableau B

Wassermann, Matefy et Sédimentation globulaire chez des lepreux.

Numéros	Age et Sexe	Atteint de	Bordet Wasserman	Matefy	Sédimentation globulaire pourcentage
1	30 H	Lèpre tuberculeuse	OOH	+	60
2	30 H	Lèpre mutilante nerveuse	HHH	+++	47
3	40 H	Lèpre mixte	HHH	++	60
4	45 H	Lèpre tégumentaire	HHH	++	54
5	[illegible]0 H	Lèpre mixte	HOH	+	56
6	40 F	Lepre tégumentaire	OOH	+	
7	3[illegible] F	Lèpre nerveuse	HHH	++	56
8	30 F	Lèpre nerveuse	HHH	++	46
9	45 F	Lèpre nerveuse	HHH	+	60
10	18 H	Lèpre tuberculeuse	HHH	++	70
11	40 H	Lèpre tuberculeuse	OOH	+++	62
12	30 H	Lèpre nerveuse	OOH	+++	64
13	28 H	Lèpre nerveuse	OOH	+++	40
14	40 H	Lèpre nerveuse	HHH	+++	50
15	36 H	Lèpre nerveuse	HHH	+++	70
16	35 H	Lepre tuberculeuse	OOH	0	66
17	40 H	Lèpre maculeuse	HHH	++	54
18	40 H	Lèpre nerveuse	OOH	0	.
19	38 H	Lèpre maculeuse	HHH	+++	
20	58 H	Lèpre nerveuse mutilante	HHH	+	62
21	25 H	Lèpre nerveuse mutilante	HHH	++	62
22	25 H	Lèpre nerveuse mutilante	OOH	+++	
23	22 H	Lèpre nerveuse	HHH	+++	
24	30 H	Lèpre mixte	HOH	+	56
25	40 H	Lèpre nerveuse mutilante	HOH	+++	71
26	20 H	Lèpre tégumentaire	HHH	++	56
27	25 H	Lèpre tégumentaire	HHH	+++	58

TABLEAU B—*fn*

Numéros	Age et Sexe	Atteint le	Bordet Wassermann	Matefy	Sédimentation globulaire pourcentage
°8	45 H	Lèpre nerveuse	HHHH	+++	3
°9	38 H	Lèpre nerveuse	HHHH	+++	50
30	13 H	Lèpre mut lante nerveuse	HOH	+	60
31	°0 H	Lèpre t berculeuse	OOH	+	65
3	50 H	Lèpre nerveuse	OOH	0	67
33	30 H	Lèpre m xte	HHHH	0	
34	15 H	Lèpre nerveuse	OOH	+++	56
35	40 H	Lèpre tuberculeuse	OOH	++	70
36	40 H	Lèpre nerveuse	HHHH	+++	56
37	50 F	Lèpre nerveuse	HHHH	+++	5
38	40 F	Lèpre tuberculeuse	OOH	+++	66
39	35 H	Lèpre nerveuse	OOH	0	57
40	°0 H	Lèpre maculeuse	HOH	+++	
41	°1 H	Lèpre m xte	HOH	++	60
4°	60 H	Lèpre m xte	HHHH	+++	
43	40 H	Lèpre m xte	HOH	+	63
44	25 H	Lèpre n r euse mut lante	HOH	++	63
45	30 H	Lèpre mixte	HOH	+,	57
46	30 H	Lèpre n rveuse mut lante	HHHH	+++	60
47	°5 F	Lèpre maculeuse	HHHH	+++	48
48	30 F	Lèpre m xte	HHHH	+	61
49	40 H	Lèpre mixte		+	
50	1° F	Lèpre tégumentaire		+++	50

A/s B Wasse mann —HHHH = rés ltat négatif HOH = pos tif fa ble OOH = pos tif fort ou complet.

Tableau C

Sédimentation globulaire chez des non lepreux

Numéros	Age et Sexe	Atteint de	Sédimentation globulaire pourcentage
1	13 F	Eczéma	40
2	42 H	Embarras gastrique	36
3	35 F	Gastrite	31
4	[illegible]0 F	Gardienne	37
5	[illegible]5 H	Chancre mou	50
6	[illegible]0 F	Femme d un [illegible]	36
7	17 H	Blennorrhagie	43
8	30 F	Ascite	26
9	2[illegible] H	Ulcération	11
10	26 H	Dyspepsie	4[illegible]
11	18 F	Bronchospirochetose	40
1[illegible]	40 F	Asthme	50
13	36 F	Dysenterie	53
14	19 F	Ulcéres	4[illegible]
15	30 F	Adénite inguinale	50
16	10 H	Epilepsie	55
17	42 H	Blennorrhagie	4[illegible]
18	35 H	Phymosis	48
19	33 H	Diabète	44
[illegible]0	59 H	Rhumatisme chronique	47

SOME HÆMATOLOGICAL AND SEROLOGICAL ASPECTS OF THE POTASSIUM IODIDE TREATMENT OF LEPROSY.

BY

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POTASSIUM IODIDE is a drug whose value in the treatment of leprosy is undoubted [Muir (1)] and we considered that an investigation of certain hæmatological and serological aspects of this treatment might prove of value in attempting to explain the mode of action of the drug

The channels which have been explored so far are these —

(1) The total and differential white blood cell counts

(2) The albumen and globulin content of the serum

(3) The lipase content of the serum

(4) The effect of varying concentrations of potassium iodide on serum *in vitro*

(5) The relationship between total white cell count and red cell sedimentation rate in patients under potassium iodide treatment

I TOTAL AND DIFFERENTIAL WHITE BLOOD COUNTS

With regard to the total and differential white cell counts it was considered essential to work out the figures in the following types of cases (*a*) Patients under treatment with drugs other than potassium iodide (*b*) Patients showing symptoms of leprous reaction with drugs other than potassium iodide (*c*) Patients under treatment with potassium iodide but not reacting (*d*) Patients showing symptoms of leprous reaction while under treatment with potassium iodide

In patients under treatment with drugs other than potassium iodide and not in the stage of leprous reaction there was no evidence of leucocytosis In a series of 14 cases the highest total count recorded was 13 650 per c mm, the lowest was 5,940 and the average was 9,560 There was no relationship between the total white cell count, the extent of leprotic involvement of the tissues, or the duration of the disease With regard to the differential count, the striking feature is the relatively low percentage of 'polymorphs'. Instead of an average 'polymorph' count of something like 70 per cent the highest recorded in our series was 66 per cent, the lowest 16 6 per cent, with an average for the series of 40 7 per cent

(a) Patients on drugs other than potassium iodide

TABLE I

Name and Stage	Total W B C.	'Poly morphs' Percentage	Lympho cytes Percentage	Duration of disease
Khirode Pal (A_1 B^1)	*11 240*	32 1	62 5	2¼ years
Deo Narayan (B)	8 125	48 7	46 1	3¼ "
Deo Narayan (B^1)	7 500	40 0	53 3	3¼
Haldar (B^1)	13 650	39 3	55 3	Indefinite
Yusuf (A_1)	10,720	24 3	64 9	12 years
H Sarkar (B^2 B^3)	13 0[illegible]0	51 3	44 6	5
Musafir (B^3)	8 125	30 4	6[illegible] 4	4¼ "
Tarak (A_1)	*6 670*	*66 0*	*28 7*	*1* year
Ramasis Singh (B^2)	9 375	16 6	76 2	2 years
Tulsi Gowala (B^1)	8 540	50 7	35 3	12 "
B L Biswas (B^1)	9 780	37 7	[illegible] 3	6 months
Jiten (A)	10 090	44 7	39 0	6 years
M L Basak (A A_2)	11 025	55 0	41 0	Indefinite
Ramasis Singh (B^2)	5 940	32 7	63 3	2 years

Note —The cases are classified according to the nomenclature proposed by Muir([?])

On the other hand the lymphocytes (combined large and small) showed a relative increase, varying from a maximum of 76 2 per cent to a minimum of 28 7 per cent, with an average for the series of 50 4 per cent (Table I)

(b) Patients showing signs of leprous reaction on treatment with drugs other than potassium iodide

TABLE II

Name and Stage	Total W L C	'Poly morphs' Percentage	Lympho cytes Percentage	Duration of disease
Bakshan Miah (B^2)	6 670	51 7	37 0	?
Kalia Bibi (B^2 B^3)	18,650	33 5	53 5	3 years
Sk Fossi Mohd (B^2 B^3)	7 920	40 6	47 0	6 "
Bhudia (B^3)	*7 605*	*57 0*	*40 4*	*1 year*

In this small series the highest total count was 18 650, the lowest 6 670, with an average for the series of 10,210 There is therefore a somewhat wider range of

maximum and minimum variations as compared with non reacting cases of the same group but the average total counts for the two series are very similar viz , 9 560 and 10 210 white cells per c mm respectively Turning to the differential counts the highest polymorph ' count was 57 0 per cent the lowest 33 5 per cent with an average for the series of 15 7 per cent In the case of the lymphocytes the corres ponding figures were—highest count 53 5 per cent lowest 37 0 per cent with an average of 11 5 per cent (Table II)

These figures do not show any great variation from the non reacting cases of the corresponding group

(c) Patients under treatment with potassium iodide but not showing signs of leprous reaction

TABLE III

Name and Stage	Total W B C	Poly morphs Percentage	Lympho cytes Percentage	Duration of disease
Yusuf (A)	8 110	19 0	72 4	1 years
Tarak (A)	20 312	58 3	36 5	1 year
Tarak (A)	10 310	41 0	39 5	1
Tulsi Gowala (B)	11 875	62 5	27 5	12 years
Tulsi Gowala (B)	8 330	45 7	48 9	12
B L Biswas (B)	13 650	45 0	27 4	6 months
Bhudia (B²)	6 980	57 3	38 0	1 year

Here the highest total count was 20 312 white cells per c mm the lowest 6 980 with an average for the series of 11 415 Contrasted with the last two groups there is a wider range between maximum and minimum counts and a slight increase in the total count over the whole series With regard to differential counts the highest polymorph ' percentage was 62 5 the lowest 19 0 with an average for the series of 47 0 per cent In the lymphocyte series the maximum and minimum variations were 72 4 and 27 4 respectively with an average of 41 5 per cent (Table III)

The highest total count was 36 250 white cells per c mm the lowest (in a case in which the reaction was rapidly being cut short by treatment) 12 710 with an average for the series of 22 710 These findings are in marked contrast to the groups already considered Turning to the differential count (*a*) the maximum percentage of polymorphs ' was 66 3 the lowest 16 0 with an average of 43 6 per cent (*b*) the corresponding figures for lymphocytes were 74 ? 30 6 and 48 9 per cent (Table IV)

(d) Patients showing leprous reaction while under treatment with potassium iodide.

TABLE IV

Name and Stage	Total W B C	'Poly morphs' Percentage	Lympho cytes Percentage	Duration of disease
Chandiram (B^2 B)	36 250	66 3	32 0	8 years
Chandiram (B^2 B^3)	33 960	35 0	63 8	8 ,,
Abdul Rahman (B^2)	23 020	63 5	31 9	5 ,,
Musahr (B^3)	23,125	34 3	60 7	4½ ,,
Ramasis Singh (B^2)	21,390	37 1	50 9	2 ,,
Ramasis Singh (B^2)	12 710	16 0	74 2	2 ,,
Jiten (B^1)	17 400	37 7	50 6	6 ,,
Jiten (B^1)	15,730	48 5	30 6	6 ,,
Saxby (B^2 B^1)	20 830	54 4	42 5	9 months

Marchoux and Bourret(3) in their observations on a single case treated with potassium iodide, report a maximum of 19,810 leucocytes per c mm at the height of the reaction there was an increase in 'polymorphs' with a decrease in the eosinophils and mononuclears In our series there were five cases with which we were able to keep in touch during the whole course of the reaction, in all of these there was an increase in 'polymorphs' at the height of the reaction, but the findings with reference to eosinophils and mononuclears were inconstant

The total leucocyte count at the height of reaction and also the rapidity with which the total white cell count rose were greatest in the most advanced cases—to quote only two cases, (i) a B^1 case in which there was an increase of 7,310 in the total leucocyte count in four days contrasting with (ii) a B^3 case in which there was an increase of 15,000 in the total count in three days

II THE ALBUMEN AND GLOBULIN CONTENT OF THE SERUM

(a) The Globulin Content of the serum in patients under Potassium Iodide Treatment

This was estimated in a series of cases using varying dilutions of serum from 1 in 25 to 1 in 1,600 and precipitating the globulin by half saturated ammonium sulphate Sera from cases under treatment with potassium iodide and from cases on drugs other than potassium iodide were used The readings were taken immediately and also after the tests had stood at room temperature for 24 hours Without going into unnecessary details, it may be stated that no striking differences in the globulin content of the sera from the two groups of

cases could be detected by this method These results were confirmed independently by Major Boyd, the Chemical Examiner to the Government of Bengal, who used a colorimetric method (the tyrosin method of Wu)

(b) The Albumen Content of the serum in patients under Potassium Iodide Treatment

The results in this investigation as in those detailed under section (2) above are largely negative The serum albumen was estimated by two different methods (a) precipitation by full saturated ammonium sulphate and (b) precipitation by Speigler's reagent The latter is an extremely delicate test solution for the presence of albumen and serum dilutions as high as 1 in 10,000 were employed The same group of cases were used as in the globulin estimations but again no striking differences could be detected

III THE LIPASE CONTENT OF THE SERUM IN PATIENTS UNDER POTASSIUM IODIDE TREATMENT

For the estimation of the lipase content of the serum, Loevenhart's(4) method was used This consists in incubating a mixture of serum and ethyl butyrate in a given dilution at 38°C for 24 hours and titrating the acidity developed with $\frac{N}{10}$ NaOH using phenolphthalein as an indicator The lipolytic power is thus represented by the number of c cs of deci normal alkali required to neutralize the fatty acid produced by the enzyme action of 1 c c blood serum on the ester The normal is between 20 and 25

A total of 42 cases was investigated Of these nine were on drugs other than potassium iodide while the remaining 33 were undergoing the iodide treatment In the nine control cases the average lipase content of the blood was 19 0 The remaining 33 cases are divisible into two groups —(a) a series of 24 cases showing no symptoms of leprous reaction, and (b) a series of nine cases who at the time of examination were in the stage of leprous reaction In the former the average lipase content of the serum was 23 2, while in the latter the corresponding figure was 20 7 It would therefore appear that the administration of potassium iodide is associated with a slight rise in the lipase content of the serum up to the time of reaction

One point emerged from this study, viz that the onset of a leprous reaction is not associated with an immediate fall in the lipase figure and it is only after this phenomenon has persisted with some severity over a period of time that the lipase figure falls

IV THE EFFECT OF VARYING CONCENTRATIONS OF POTASSIUM IODIDE ON SERUM *in vitro*

Aqueous solutions of potassium iodide of 2 per cent, 5 per cent and 10 per cent strengths were taken equal quantities of serum and of the three

strengths of potassium iodide were put up The sera were obtained both from cases on potassium iodide and from cases on drugs other than potassium iodide they were tested both fresh and also after inactivation in a water bath at 56°C for half an hour Some of the tests were put up at room temperature others were kept for one hour in a water bath at 54°C to 56°C all were allowed to stand overnight before the final readings were taken The results were consistently negative and all the tubes remained absolutely clear

V THE RELATIONSHIP BETWEEN TOTAL WHITE CELL COUNT AND RED CELL SEDIMENTATION RATE IN PATIENTS UNDER POTASSIUM IODIDE TREATMENT

Twenty cases under varying doses of potassium iodide were tested to try to elucidate a possible relationship between the white cell count and the red cell sedimentation rate The following facts emerged from this enquiry —

(a) In non reacting cases there is no parallelism between red cell sedimentation rate and total white cell count—cases showing approximately the same white cell count may show a deviation in their respective sedimentation rates amounting to over 100 per cent

(b) In the stage of reaction, a high total white cell count and a rapid sedimentation rate are usually associated

(c) The total white cell count assumes its pre reaction level much more rapidly than does the red cell sedimentation rate

SUMMARY AND CONCLUSIONS

(1) In non reacting cases of leprosy, the total white cell count lies within normal limits the differential counts show a deviation from the normal in that the 'polymorphs' are diminished while the lymphocytes are relatively increased There is no absolute relationship between these findings and the type, stage or duration of the disease

(2) Reaction producing agents tend to cause a leucocytosis and this phenomenon is most marked where the reaction is dramatic and abrupt in onset such as occurs following administration of potassium iodide

(3) The administration of potassium iodide *per se* does not cause a leucocytosis in the absence of a leprous reaction

(4) There is no appreciable change in the albumen or globulin contents of the serum due directly to the action of potassium iodide

(5) Sera of patients under treatment with potassium iodide do not give any precipitation reaction with varying concentrations of the drug

(6) While leucocytosis and acceleration of sedimentation rate are commonly found in association, the relationship is not a constant one moreover, the alteration in the leucocytic count which occurs with the onset of reaction is a much more acute phenomenon than is the change in the sedimentation rate The leucocytic

count also tends more rapidly to assume its pre reaction level than does the sedimentation rate

(7) There is a fall in the lipase content of the blood during the latter stages of prolonged reactions

REFERENCES

(1) MUIR E. (1927) Trans 7th Congress F E A T M
(2) *Idem* (19[illegible]) *Lancet* Vol I No 24 January
(3) MARCHOUX E., and BOURRET G (1908) *Bull Soc Path Exot* 1 pp 347—350
(4) WHIPPLE (1913) *Bull Johns Hopkins Hospital* Vol. XXIV p 357

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SUBSIDIARY USES OF POTASSIUM IODIDE IN LEPROSY

BY

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BESIDES the use of potassium iodide in the treatment of leprosy there are certain other subsidiary uses which are of considerable importance, viz :—

(1) In making a diagnosis in doubtful cases and contacts in whom there are no clinical signs

(2) As a prophylactic in contacts in whom no clinical signs are present

(3) In testing the reality of cure in cases in which all active clinical signs have disappeared

(1) *In diagnosing doubtful cases and contacts*

Thirty three children of leper parents in the homes for healthy children connected with the Purulia Leper Asylum were given orally increasing doses of potassium iodide The results are recorded in tabular form (Tables I and II) Of the thirty three, 17 were boys and 16 girls

Taking first the 17 boys (Table I), 15 showed signs suspicious of leprosy either at the time of administration of iodide or at some period previously, but it will be noticed that these signs would not have been counted suspicious of leprosy unless the children had been in contact with lepers The suspicious signs were depigmented patches and slight thickening of nerves, especially the right ulnar, and dryness of the skin After administration of iodide, the signs diagnostic of leprosy were rises of temperature and tenderness of nerve trunks Of the 15 boys showing suspicious signs before iodide 11 gave positive signs after iodide i e, both rises of temperature and tenderness of nerves, 1 was negative and 3 were counted still doubtful as they showed slight rises of temperature but no nerve tenderness Of the two who originally showed no signs, both were distinctly positive after iodide

Of the 16 girls (Table II) 8 had suspicious signs similar to those of the boys and of these eight cases, the iodide showed two positive, one doubtful and five negative Of the 8 who showed no signs before iodide, 4 gave suspicious signs and 4 were negative after administration

The larger number of positives among the boys as compared with the girls is probably due chiefly to two causes (a) The boys are allowed to run about much

more freely and are therefore more liable to become infected by their leper parents seeing that the children's home is not very far distant from the leper asylum (b) The average age of the boys is 8 that of the girls 14 6 The latter have been living in sanitary surroundings away from infection and have been well cared for during a longer time

While therefore we do not claim to have an absolute test of infection we consider that we have in potassium iodide a test which is of very great value and one which may be used to determine the presence or absence of infection in suspicious cases and in contacts It goes to prove that the infection is present at first in most children who have been in contact with infectious leprous parents relations, etc and it is an interesting fact that most of these children who are brought up in a home under favourable circumstances never develop the disease and that such slight signs as do appear at first from time to time become entirely absent as the children grow up The highest dose given was 240 grains and that only to the older children the maximum with the younger children was 120 and with the very small ones 60 grains It must be remembered that generally speaking children stand iodides better than adults

(2) *Potassium iodide as a prophylactic in contacts in whom diagnostic clinical signs are present*

In the series of cases at Purulia referred to above it was found in most cases that the fever and nerve tenderness caused by iodides disappeared and that after the larger doses of 120 and 240 grains had been reached and repeated once or twice no further rise of temperature or nerve tenderness occurred The presumption is that the iodide not only showed up the presence of disease by causing these signs but also helped to clear up the disease in the affected parts We consider that we are justified in saying that to a certain extent iodide is a prophylactic in the sense that it at least partially clears up lesions which could not have been definitely diagnosed either clinically or bacteriologically without the use of iodide We do not claim however that in every case the periodic administration of iodide will without fail prevent the occurrence of leprosy

(3) *In testing the reality of cure in cases in whom all active clinical signs of leprosy have disappeared*

It follows as a natural inference from the first section of this paper that if iodide can show up leprosy in its earliest stage when signs sufficient of themselves to determine a positive diagnosis have not yet appeared, the same drug will be able also to show up existing remains of leprosy in cases in which with or without treatment the signs of active disease have disappeared We have found iodide most useful in this direction both in revealing unsuspected lesions and afterwards in clearing them up Again we do not claim any infallibility for this test When it is positive it is extremely useful when it is negative there is still always the possibility that there are lesions in the body which even massive doses of iodide have failed to affect

TABLE I

BOYS

Name	Age	Original clinical signs		Max. dose in grains	K I in grains	Reaction				Result
		Skin	Nerve			Rise of temperature	Duration	Skin	Nerve	
1 Gabriel	14	Depigmented area right cheek (not leprous)	Right ulnar nerve slightly thickened	200	After 4	10° 2°			Ulnar neuritis	+ ve.
2 John II	1°	Small depigmented spots front of chest large depigmented area spine left scapula suspicious dryness left forearm	Right ulnar nerve slightly thickened.	°00	After 200	100·2°			Right ulnar neuritis after 60 grs	+ ve.
3 Philmon	10	Suspicious depigmented patch over left malar bone depigmented area near right ala of nose		1 0						— ve
4 Bartholemew	11		Slight thickening right ulnar nerve	150	After 7½	99°			Tenderness both ulnar nerve after 60 grs	+ ve
5 Abosalem	10	Suspicious depigmented patch right cheek.	Right ulnar nerve slightly thickened.	120	After 5 120	99° 101 5° (Thereafter two small rises.)			Both peroneal nerves became tender after 1°0 grs Thereafter no more nerve trouble with 1°0 grs.	+ ve.

6 Mohendra	10	Susp c ous dep g mented areas both cheeks and nose	R ght ulnar nerve sl ghtly th ckened	1"0	After 30 1.0 seventh 1"0 grs dose	99° 99° 99 4°	2 days		After seventh 1"0 grs. dose tender ness of ulnars and peroneals.	+ ve.
7 Sikhoria	10	Dep gmented both cheeks and nose	R ght ulnar nerve sl ghtly thickened Also right peroneal nerve	1"0	After 30 1"0	99 4° 100°	2 days			?
8 Rupchand	9	Dep gmented both cheeks, small de-p gmented spot back of left fore-arm (Found pos t ve in June by Dr Santra, i.e leprosy but not bact. pos tive)	R ght ulnar nerve sl ghtly th ckened.	180	After "½ 5 10 "0 180	99° 99 4° 99 "° 99·2° 99 4°	1 day 1 day 1 day 1 day 3 days		After 180 grs. tenderness ulnar and peroneal nerves.	+ ve
9 Benjamin	8	Very sl ghtly de p gmented patch both cheeks		1 0	After 2½ 100	99·"° 99 6°	1 day " days		100° after 100 grs. left peroneal tender	+ ve
10 Probhudhone	7	Slight dryness of sk n over both sh ns.	R ght ulnar nerve s ghtly thickened	5	After "0 7.	99 2° 10 4°	1 day 1 day		N I	?
11 Soleman	7		Both peron al nerves s ghtly th ckened	5	After 10 75	99° 100 "°			After 75 grs. tenderness both peroneals and left ulnars.	+ ve
12 Mothi	7		R ght ulnar nerve dist nctly thick ened.	75	After "0 75 5	99 "° 10 ° No n ore react		75 grs. slight erythema, tenderness of sk n on outer s de of left forearm	Right ulnar tender after 1"0 grs.	+ ve.

TABLE I—*concld*

Name	Age.	ORIGINAL CLINICAL SIGNS		Max dose in grains.	K I in grains	REACTION				RESULT
		Skin	Nerve.			Rise of temperature	Duration	Skin	Nerve.	
13 Moshi	6	Slight trace of depigmentation on outer side right calf Tiny depigmented area between back of shoulders	Right peroneal nerve thickened	75	After 20 60 75	99° 99 2° 99°	2 days			?
14 Parnotas	6	(Marked as suspicious case in June by Dr Santra)		75	After 21 20 75 75 75	99° 100 2° 100° 100 6° 99 8°	2 days 2 days		After both peroneals tender after sixth dose of 75 grs	+ ve
15 John III	5	Tiny depigmented areas over lower part right shin (Marked as suspicious case in June by Dr Santra)		60	After 21 40 60 Then 60	98 8 99° 104° N R	[illegible] days		Tenderness of ulnar and peroneal nerves	+ ve
16 Ismail	4			60	After 60 7th × 60	9[illegible]° 100·2	2 days		Tenderness of both ulnar nerves	+ ve.
17 Kalendra	4	Had recently left patients quarters and gone to healthy children's home		60	3rd 60 iodide rash and secondary infection 7 days = to then 60—5 days	10[illegible]°			After 60 grs. tenderness of ulnar and peroneal nerves for several days	+ ve

Table II

Girls

Name.	Age.	Original clinical signs.		Max dose in gram	K. I. in grains.	Reaction			Result.
		Skin	Nerve			Rise of temperature	Duration.	Nerve.	
1 Samali	25			240	After 240 " 240 " 240	99° 100 2° 99·2°			?
2 Kusum	18			240					— ve.
3 Premika	17			240					— ve
4 Santimoni	17			240					?
5 Chandamukhi	18			40					— ve.
6 Soroni	16	Pigmented patch above left angle of mouth.		240	After 240 iodide rash After 240 two or three times.	99 8° 99°			?
7 Dayamoni	16			240					— ve
8 Binodini	16	Tiny depigmented spot left cheek noticed since 1923		240					— ve

TABLE II—*concld*

Name	Age	Original clinical signs		Max. dose in grain	K I in grains.	Reaction			Result
		Skin.	Nerve			Rise of tempera ture	*Duration*	Nerve.	
9 Naomi	15			240					— ve
10 Promodini	13			175					?
11 Johanna	13	Trace of depigmented over left malar bone		175					— ve
12 Dharmika	10	Glossy skin over both shins	Slight thickening of left ulnar nerve	120	After 40 ,, 120 ,, 120 , 180	99° 100 6° 100° 99°		40 grs sl ght tenderness right ulner nerve but no more after	+ ve
13 Mohini	11		Left ulnar nerve slightly thickened	150					?
14 Sukumari	11	Tiny dep gmented spot outer side right forearm. Irregular depigmented patch on inner side left forearm	Right ulnar nerve slightly thickened	150	After 10 , 150 , 150	99·2° 99 5° 101°	2 days 3 days	20 grs.—slight tender Rigl t ulnar nerve but no more after	+ ve
15 Rebecca	10		Left ulnar nerve slightly thickened	180	After 7½ ,, 180	100 2° 99°			?
16 Kristokaruna	9	Anhydros s both feet. Dryness of skin	Left ulnar nerve slightly thickened.	100					— ve

LEPER SETTLEMENT DEVELOPMENT

BY

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INTRODUCTION

As a basis for this paper I am using experience gained in organizing and developing the Lady Willingdon Leper Settlement, Chingleput In some ways this experience has been unique, for we had new buildings and an old population and somehow the two had to be assimilated It offers a good field for the study of leper settlement development and the use of such institutions for the study and treatment of leprosy We hope we have learned something from the many problems arising in the process of organizing and developing and by passing on some of our experience we trust we might thereby be doing a service to others who might be contemplating similar schemes

What is now the Lady Willingdon Leper Settlement can be traced back to 1841 when apparently a leper asylum was founded in Madras Latterly this asylum was at Royapuram a northern suburb, and surrounded by a suburban population Government, under whose charge it was, decided to move the asylum to a less populous district A new settlement was erected at Tirumani which is some three miles from Chingleput—a town of 12 000 inhabitants, 35 miles south of Madras, on the main line of the South Indian Railway The Royapuram population of some 450 was transferred to the settlement at Tirumani on 30th April, 1925

The Government made an agreement with the U F Church of Scotland Mission, whereby the entire management was handed over to the Mission for a period of five years in the first instance The Mission has appointed a European superintendent, a medical officer and a matron, and the Government pays all expenses except the superintendent's salary

ORGANIZATION

Lay out —The area is divided into three zones—clean, neutral, and tainted The staff residential quarters are in the clean zone In the neutral zone are the administrative blocks, viz , general and medical, and removed at a little distance are two observation blocks The tainted zone is divided into two parts—male and

female—separated by a compound wall Along this wall are the common buildings, viz dispensary, hospital boarding school and recreation hall On the female side there are 13 separate blocks each with two rooms and each housing six patients On the male side there are 58 similar blocks and four blocks for Anglo Indian families

Population —At present there is accommodation for 480 There are 348 adult males 86 adult females 24 boys and 19 girls Divided on a basis of religion we have 80 per cent Hindus 5 per cent Mohammedans, 15 per cent Christians

Of our population an undue percentage are of the burnt out beggar type and unfortunately at the beginning of our regime these people set the tone to the whole place For some time it was very difficult to keep the better type of leper patient, till I was persuaded that it was almost impossible to retain a youth between the ages of 16 and 22 Time and time again such a patient with the disease in its early stages would be admitted to the settlement and abscond within a few days Lately we have altered the housing arrangements of our patients We have practically divided the settlement into two reserving an area of 32 blocks on the male side for the advanced types and the remaining blocks separated from the former area by a main road are reserved for the earlier type of case Of the 86 adult Indian females, 48 would be classed as A_2

Treatment Difficulties —It is essential that all treatment should be regular The present position of the law as regards segregation does not allow us to restrain the movements of lepers Our experience has been that there is far too much coming and going For instance last calendar year, we had 760 discharges and 916 ad missions While much of this movement takes place among the beggar class very frequently we have lost in this way some of our best and most hopeful patients We have only about 100 patients who have taken treatment with any degree of regularity over a period of one year We started with only 13 per cent of the adult population taking injections—all the children must take treatment For some time this figure was practically stationary, but with the beneficial results of treat ment showing themselves the percentage has lately increased until now we have some 33 per cent of the population on active treatment Since taking over the settlement 27 cases have become symptom free This number ought to have been and would have been much higher were it not for the fact already mentioned that many left in the last stage of their treatment In addition 13 left us while under their final period of observation Our experience is that the women are more reluctant than the men to take treatment

Medical —When we took over the place in 1925 we had to re chart the whole population We have now outline diagrams of each patient s body showing the various lesions and details of the history of the case Re charting is regularly carried out

When we began work in 1925 the treatment adopted was the subcutaneous injection of ethyl esters of hydnocarpus oil with the addition of four per cent creosote

commencing with half a c c and increasing to ten c cs Later we began to use pure hydnocarpus wightiana oil as it had been stated that the latter was as effective as the ester and was considerably cheaper The patients complained of more pain under this form of treatment than with the esters but it was found that much of the pain was due to lack of sufficient exercise Our experience confirms the general view that under hydnocarpus oil treatment exercise is a vital factor We found that the patients most responsive to the treatment were they who were leading energetic lives, and now every patient certified as being fit for it is required to do two hours light manual work per day There is great reluctance on the part of the patients in carrying out the rule It might be noted that the oil must be prepared from fresh seeds and when the tin is opened all the contents must be emptied into glass stoppered bottles and stored in the dark Many are still on ester treatment and although it is more costly than the oil in my experience it gives quicker and better results We now treat skin cases with oil and nerve cases with esters because we found that severe reactions occurred much more frequently among skin cases than among nerve cases on ester treatment

Hydnocarpus wightiana oil and its derivatives continue to be the basis of our treatment but we are now trying the effect of potassium iodide on about 100 patients As yet I have no information to put before the Congress as to the results of this treatment

Considering the serious reactions which sometimes followed the injection of organic arsenicals in the treatment of associated syphilis in lepers, I can report excellent results obtained from the use of Hg 33 now on the market as Avenyl A 0 25 per cent solution of the drug in hydnocarpus oil with four per cent creosote added is used Some 18 months ago Dr Muir on a visit to the Settlement, brought a quantity of the drug dissolved in hydnocarpus oil and in its use the results were exceedingly encouraging I am now using it in all cases with a positive Wassermann reaction or a positive Kahn Every patient has his blood tested before beginning anti leprotic treatment and if found positive is at once started on Avenyl The value of such a drug can be appreciated when I state that 55 per cent of our patients have either a positive Wassermann reaction or a positive Kahn

We employ the Kahn test for the detection of syphilis It is exceedingly simple and requires very little apparatus thus making it most suitable for employment in small laboratories such as are connected with leper settlements or asylums Dr Muir kindly supplies me with the antigen

Diet —In the treatment of leprosy as in all diseases diet is an important element At first we took over the diet which had been given in Madras Being situated in a rice eating country this was based on a full ration of 24 ounces of raw rice per head per day This is more than a healthy man in active employment can profitably consume We found that a good many of the patients were disposing of surplus rations to the villagers—a very objectionable practice In

consultation with Col McCarrison and the Surgeon General a new diet scale has been worked out and it is as follows —

Ordinary diet for lepers not under active treatment

(1)	Rice Ragi Cholum or Cumbu (patients to have choice)	18 ozs
(2)	Dhall	6
(3)	Salt	$\frac{3}{4}$ oz
(4)	Ghee	$\frac{1}{2}$
(5)	Tamarind	$\frac{1}{2}$
(6)	Curry powder	$\frac{1}{4}$
(7)	Onions	$\frac{1}{2}$
(8)	Vegetables	8 ozs

Two ounces of dhall may be replaced twice a week by 4 ounces of mutton

Recognizing that dairy products are essential elements in all diets and especially for lepers under active treatment at the time of writing arrangements are being discussed whereby it is hoped that an additional quart of milk per day will be given to each leper under such treatment

Learning from our experience of the former scale of diet if this is approved we shall attempt to insist upon the milk being drunk at the time of delivery in the presence of one of the staff

Suggestions for Future Settlements

I have now briefly covered the more important items of our experience and I might be allowed to conclude the paper by outlining an ideal settlement

(1) *Separation of the Sexes* —I am doubtful whether settlements should be built to accommodate male and female lepers Whatever precautions may be taken it seems to be quite impossible to keep the one sex from the other In any case most settlements do attempt to separate male and female and I think this could be more effectively secured by building quite distinct places separated from each other by some little distance At first sight it might appear that such an arrangei both lepers coming In fact we have nc 15—and on medical grounds these should not be allowed to live together for the following reasons —

(*a*) Leprosy is of two types—skin and nerve—and the person with the nerve type may develop the skin type or vice versa and so become a mixed type In the housing arrangements of our settlement we make a definite attempt to keep not only the two main types in separate areas but also types with differing degrees of intensity are housed in blocks according to their medical classification

In the case of man and wife living together each with a different type of leprosy there is a real danger of the non infectious type becoming infectious Even if man and wife were of the same type, e g , skin, but of differing degrees of intensity there is every probability that the partner with the light infection would become heavily infected

(*b*) In the case of leper women whether of the skin or nerve type, child bearing aggravates the disease

(*c*) It can be accepted that children in their infancy are very susceptible to the disease In the case of lepers bearing children theoretically the child should be taken away at birth, but in practice this is almost impossible A child must be left with its mother for a period of at least one month and only then can the separa tion be made During this period of continuous and close contact, it is very probable that the child will contract the disease though it may not show itself until in later years

If a determined effort is to be made to stamp out leprosy, one of the most essential prophylactic measures is to reduce to a minimum the possibility of lepers bearing children and this can be done only by a separation of the sexes

(2) *Burnt out Cases* —Separate settlements should be maintained for burnt out cases These are usually of the beggar type and lazy dirty, and quarrelsome Besides this, their deformities are not attractive! If they are in a settle ment in large numbers they are a serious problem to the management They seem to be able to set the tone to the place and the sight of them repels and frightens the more amenable cases with the result that a settlement with this type tends to become largely for this type Such a separate asylum might be built within reasonable distance of the treatment centre but separated therefrom This arrangement would enable the one management to be responsible for both places and to separate their cases at the time of admission

(3) *Children* —Almost ten per cent of our population are children under 15 In most cases the children, when they come to us, are not heavily infected, yet we do not see the improvement in their condition which we might reasonably expect This may be due to their being allowed to move about freely in the settlement and perhaps thus their progress is retarded We have a board- ing school in which they live but in the nature of things, although we try to do so, it is impossible to restrict their movements to the vicinity of this school The child leper of to-day becomes the advanced case of leprosy of to-morrow

In the planning of any settlement it would be desirable to have a separate spacious detached area for children only and they should not be allowed to move among the more advanced cases

In addition to these definite divisions, we are assuming that there will be obser- vation blocks and a home for untainted children

General Suggestions

(1) If the problem of leprosy is to be successfully dealt with institutions with these divisions will require to be multiplied over the land Such places would naturally become the centre of a geographical district Dispensaries or skin clinics could be established in the surrounding area and these would be the feeders for the central institution At present we ourselves largely pick up our cases as casuals at the gate and this is a most unsatisfactory method

(2) If such comprehensive institutions multiply, it is a question to be considered whether it would not be advisable so to modify the Indian Leper Act as amended up to 1920, that lepers within the area actually covered by the institution would be obliged to remain there till they had permission to leave This permission could be given in special cases for reasons other than a cure Such a modification might make segregation less distasteful At present there is no power given to retain a patient under treatment until such time as he might be considered symptom free, and time and time again in our experience before their course was completed, hopeful patients have left us, not to return A stay of another three or four months might have meant their discharge as symptom free and their return to ordinary life, whereas by voluntarily going almost assuredly they will develop into more advanced cases and become a menace to the public

(3) At the present, treatment and pathology are undoubtedly the aspects of leprosy demanding study and attention After two years' experience in a comparatively new place with a standing population of more or less 150 I cannot say that I am satisfied with my medical results Some of the reasons for such a situation have already been detailed and the limitations under the present legal conditions are obvious but I also feel that so far as treatment is concerned the last word has yet to be said

With these model institutions there should go definite facilities for research on the disease The obvious line of research in such a place would be in connection with the treatment and pathology of the disease For this purpose one medical officer at least would be required who was not burdened with administrative duties With further study of the pathology of the disease it might be expected that treatment would move forward to a new stage and this above all is a thing to be desired The present treatment is a course extending to as much as two years and while good results are forthcoming yet very few patients are prepared to face a continuous course over this length of time Even if they start quite cheerfully it seems to be difficult to maintain hope to the end and one finds that the cheerful co operation of the patient is essential May be it is for this reason that we find in our own experience that the better educated people are our best patients They understand the situation better than illiterates and can give you the necessary co operation in spirit

(4) Such settlements will provide ample material for the study of the disease and, if equipped with a well appointed laboratory, could easily serve a very useful purpose not only in research work itself but also as training centres At the

present moment the ordinary practitioner frequently finds difficulty in diagnosing cases in the early stages and such a place would provide facilities for the training of doctors in diagnosis and differential diagnosis It has to be considered whether skin diseases in general and leprosy in particular has the part in the present medical school curriculum which their importance would seem to demand for this country Should not every student passing through our medical colleges be compelled to take a short course in leprosy and have a prospect of at least one question on the subject in his final paper ?

THE PROPAGANDA TREATMENT SURVEY CENTRE AS A MEANS OF DEALING WITH LEPROSY

BY

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THE old method of dealing with leprosy was to seek to segregate, either voluntarily or by force such lepers as were most conspicuous and who, by the nature of their lesions, force their notice on the public Such lepers are of two types —(1) marked skin or nodular cases with thickened leonine features, and (2) secondary nerve cases with disfigured and disabled hands and feet Paupers of these two types, i e, those who beg or have no ostensible means of livelihood are the more conspicuous, and therefore attract the notice of the public most, while better class patients who support themselves or are supported by their friends or relatives naturally hide themselves as much as possible from the public, but, as many of them are engaged in some vocation or live with their families they tend to spread the disease much more than paupers do As the majority of lepers in these two stages (the third and fourth) are not paupers, and as there is not sufficient accommodation in leper institutions to segregate even those who are paupers, the great majority of them remain unsegregated But even supposing it were possible to segregate all conspicuous lepers as has been done in the Philippines, the root of the matter would not be reached for the inconspicuous lepers, those in the first and second stages, would still remain unsegregated and those in the second stage would continue to spread the disease to others

Clearly if any effective method is to be evolved for stamping out leprosy, it is necessary first of all to have a clear understanding on the following points —

(1) *What are the most highly endemic areas?* Large numbers of begging lepers are found in towns, but most of them belong originally to villages and go to the wealthier town in hope of alms If the problem is to be dealt with, it must be in the villages The 1921 census figures show that certain districts are more leprous than others and within these districts certain thanas and groups of thanas show a higher concentration of lepers These figures are however collected by untrained enumerators who are only capable of recognizing the more obvious cases It is

necessary therefore to conduct a skilled survey so as to check the unskilled census and thus find out as far as possible the actual numbers and in what areas and among what classes of the people high endemicity obtains

(2) *The second point which requires clearing up is Why is there more leprosy in certain areas and among certain classes of the community?*

(3) The third point is *Is the disease on the increase and is it spreading from other areas or to other areas?*

(4) Lastly *What means can be taken to deal with the disease in highly endemic areas?*

The method adopted was as follows —

A medical officer was appointed who had worked under the writer for over six years and who had acquired a very extensive knowledge of leprosy Under him were placed four assistants who had had a shorter period of training One of the *most highly endemic districts was chosen and a beginning was made in a thana* within easy reach of the principal town of the district

The question was how to carry out the survey Any show of force would at once have frightened the people and led them to hide their disease We began by giving lantern lectures in the villages at night showing how to recognize leprosy and how to prevent it and also that there is a remedy for it Those afflicted asked for treatment and a dispensary was started where 300 patients were attending within three weeks of beginning many of them coming from villages 10 or 15 miles distant Under such circumstances the survey was easily carried out as in every village that was entered there were eager patients willing to help The survey showed about four times as many lepers as the unskilled census had shown but as the survey of the thana only occupied a month it was clear that nothing like the whole number had been found I myself visited a village of 200 inhabitants in which our officers had found ten lepers Within a quarter of an hour we had found two more

When the survey officers passed on to another thana of the same district a doctor who had been deputed by the District Board and who had been trained during the month of survey, carried on the dispensary As the names of all patients attending are recorded in the dispensary it is likely that in this way the survey will gradually approach completion as fresh cases are constantly coming for treatment whose names are not yet recorded and as the dispensary doctor will follow up previously unrecorded infectious cases to their villages and houses and examine contacts

The survey officers are spending two or three months in each of the provinces of India where leprosy is rife Their survey though brief will, it is hoped, give some clear idea of the frequency of leprosy in each province and demonstrate the lines upon which it may be combated

It is also hoped that similar bands of survey workers will be appointed in each province to carry on the survey work once begun and that in each district this survey may be followed by the establishment of two or more leprosy clinics Such

clinics where carried on conscientiously by trained doctors are very popular and are not only centres for treatment but serve to train village doctors and to teach the villagers how leprosy may be prevented Two such thana dispensaries will in fact act as models for the whole district, especially if they are carefully superintended by a provincial leprosy officer

Another fact brought out by the survey is that leprosy is being spread from the more endemic to the less endemic districts One of the most leprous areas of Bengal is the Sadar sub division of the Bankura district This is also frequently a famine area and in years of scarcity the labouring classes migrate to the surrounding districts for work In several instances we have traced the incidence of leprosy in distant regions to labourers belonging to such castes as the Bawris and Bagdis who have migrated from Bankura I heard lately of similar happenings in the Malda district, once comparatively free from leprosy, but now invaded by labourers from the highly leprous Santhal Parganas on the other side of the Ganges who are thus spreading the disease

Many other interesting facts and statistics are emerging from the survey work but the above is sufficient to show that we have in the P T S (Propaganda Treatment Survey) centres a means of tackling leprosy which is going to the root of the problem

In the thana referred to above, where the survey was begun in the Bankura district 919 cases of leprosy were found Of these, 167 were early, non infectious cases which could only be diagnosed clinically Such cases yield rapidly to treatment If such cases can even be arrested in their progress let alone cured the source of infection will be cut off from the next generation and leprosy will tend to die out rapidly

Another fact which recommends the P T S centre is its comparative inexpensiveness as compared with the old plan of segregation Apart from the expense of drugs there is only the cost to the district of the salaries of two doctors and two compounders under Rs 4 000 a year and the cost to the province of some 10 or 12 thousand a year for a period of five years during which time the survey can be initiated and dispensaries started in every district Once begun the work of supervision can be carried on by a single leprosy expert for the province If this is compared with the expense of a leper institution like that at Gobra Calcutta, which, though run on economical lines, costs between 60 and 70 thousand rupees a year for 150 patients or Rs 433 a year per head in addition to the huge initial capital expense on land and buildings it will be obvious that if the leprosy problem is to be dealt with efficiently reliance must be placed on the P T S centre or some similar method

It is not intended to decry segregation institutions such as asylums hospitals, homes or settlements for caring for segregating and treating lepers These are useful and necessary but the contention of this paper is that such institutions alone do not reach the root of the problem and if leprosy is to be eradicated some such organization as is proposed above must be adopted

DISCUSSION

Dr O Schobl (Philippine Islands) Referred to some precipitation tests which had been carried out by himself in Manila and asked if any work along these lines had been done in India

Dr R S Donaldson (Madras) So far as my experience has gone in the treatment of potassium iodide I am of opinion that those cases, which have reactions either in the form of the appearance of fresh nodules and the swelling up of old ones, or in the form of nerve reactions, where the nerves become painful and swollen, ought to have facilities for treatment with potassium antimony tartrate or adrenalin, as the case may be, at once The pain which is present in these reactions is often very severe and if the patient cannot get what one might call the antidote at once, he may not only have great suffering but his reaction becomes increasingly difficult to control the longer the treatment is delayed It is for this reason I would advocate that in all propaganda-treatment centres where iodide treatment is given, a doctor ought to be in attendance daily so that these sufferers can receive the necessary relieving injections

Dr Gupta (Bengal) Emphasized the importance of the training of medical students in leprosy as patients preferred to be treated by their own doctors rather than go to leprosy clinics

Capt P Ganguli (Bengal) The idea that the medical college students should have proper training in the diagnosis and treatment of lepers before they pass out is very good indeed, but the difficulty is that persons competent to give this training are rather limited [illegible]
course of 15 days [illegible]
medical man m[illegible]
There are already a number of trained medical men who are carrying out the latest methods of treatment in the outlying stations of this country It would be well if the delegates spread this information all over the world

I consider that the propaganda treatment survey centres as a means of dealing with leprosy are more useful than the leper settlement method, because apart from the question of cost, which a poor country like India can ill afford the natural instinct of leper patients is to conceal their disease rather than to give up their earnings and avocations, and leave their families to starve in order to live in the leper settlements Besides, the cost of sufficient leper settlements would be prohibitive if considered from the point of view of the very large number of lepers living in India If, however these people come to know from propaganda work that there is a cure for this disease and that the *attendance in the treatment* centres will not involve giving up their avocations and living apart from their families, they will not spare any pains or any reasonable expenditure of money in order to attend these centres and obey the instructions given to them for minimizing the spread of the disease

Major Labernadie's serological tests are interesting but the precipitation test with distilled water as related by the chairman of this meeting will, I am afraid, not help us much in the detection of early cases Besides, this test has been found positive in some other diseases, common in Bengal, such as kala azar It is positive in some long standing chronic skin diseases with which leprosy may be confounded The precipitation in this test depends on the increase of globulin in the serum The anti-bodies are supposed

to reside in the euglobulin factor of the serum and hence we find that the excess of euglobulin is precipitated by the addition of distilled water to the serum of kala azar and long standing malaria cases Even in diseases of short duration such as pneumonia, where anti bodies are developed in a short time, this test gives a positive reaction I do not consider that this test will be an advance towards the diagnosis of leprosy, at least in Bengal, where various other diseases give positive reactions to the precipitation test

Dr Isaac Santra (India) (1) Dr Donaldson raises the question of the treatment of severe reactions in propaganda treatment survey centres This is very important because the survey party has opened seven centres in eight months and about 2 879 cases are treated in these centres

In my experience of both asylum work and propaganda treatment survey work, I find that severe reactions are more common in asylums than in the village centres In my second and third visit to these centres I have found the number of patients increasing and treatment getting more and more popular Patients when they do not get fever know that their disease does not improve I have heard many patients complaining against the propaganda treatment survey doctor at their not getting fever Thus reaction is a point in favour of centres Of course when the doctor has not sufficient experience to calculate the dose severe reactions are produced, but we do not have such cases in any of our centres

Dr Isabel Kerr (Hyderabad, Deccan) Mentioned the effect of treatment in attracting patients When treatment was first adopted at Dichpalli leper home there were 120 admissions within a month

NOTE ON LEPROSY

BY

D A D'MONTE, M D,

Bombay

LEPROSY is one of the oldest known diseases and has proved itself most puzzling to science Even our present-day knowledge of it is far from definite or conclusive The incubation period for instance until very recently was practically unknown We have so far been able neither to cultivate the bacillus of leprosy nor to produce the disease artificially even in the human body

As a result of my personal observation extending for well over a quarter of a century as a private practitioner as well as in my capacities as a member of the Ack leper asylum at Matunga and the honorary secretary of the Allbless leper home Trombay, I cannot vouch for the disease being contagious as there is not a single instance of any of the attendants, nurses or doctors ever contracting it through their having lived in closest proximity to lepers and through having attended them generally

The present medical officer of the asylum at Trombay, when he took charge of it about 20 years ago, drew my attention to two boys, aged 14 and 16 respectively, who were not lepers but who were allowed to continue as inmates of the asylum just because they were born there of leprous parents both of whom died in the asylum

As soon as the error was discovered, the boys were of course sent away Now it is worthy of serious attention, that both the parents of these boys suffered from and died of leprosy, that the boys exposed themselves to risks of contagion by living with other leper inmates of the home sleeping in their cots eating out of their plates and perhaps [illegible] but did not up till the time of [illegible] disease [illegible] home at Trombay, who has been washing the clothes, bed sheets, blankets, etc, for the lepers since the last 30 years, but does not yet exhibit any sign of the disease True the incubation period is long and the development of the disease slow, but 30 years ought certainly to be long enough for any disease to manifest itself

The existence of a positive family history in either one or both the parents does not necessarily favour the development of the disease in their children, as no greater predisposition is observed in these than in those with a negative family history In fact, according to some observers, heredity has little or nothing to do

with the spread of leprosy and indeed the children born of leprous parents seem to acquire a certain amount of immunity against the disease

Infection is possible through contact of broken surfaces Whether the bacilli can be carried from a leprous sore to a healthy wound by handling a pipe cigar, knife or any other intermediate body I am not in a position to say

The bacillus appears to be very weak and not able to thrive outside a human body

The incidence of leprosy does not seem to have a great deal to do with intemperance immorality unhygienic living deficient food and so on except in so far as these conditions lower our natural power of resistance and lay us open to any kind of contagion Some authorities Hutchinson amongst them talk of the disease having a *de novo* origin While I am not prepared to accept this theory, I must state that I have noticed the progress of the disease having been arrested without any active treatment How can one account for the sudden disappearance of all signs—clinical as well as bacteriological—in some sufferers without any kind of treatment ?

We already acknowledge that Hansen s bacillus is the cause of leprosy, but where the bacillus comes from and how it enters the body are still matters for speculation

The chief consideration for us is early diagnosis of the disease long before it becomes infectious and its cure The Provincial Leprosy Committee in India are at present busy training medical men in the diagnosis and the latest methods of treatment Bombay is specially fortunate in this regard and with the donation of a lakh of rupees by a generous Indian a special clinic is to be shortly established in the Haffkine Institute and King Edward Memorial Hospital at Parel In addition to this clinics are being opened in various other districts with a trained medical man in charge of each clinic

Caju fruit has just been credited with curative properties against leprosy One case has just been reported from Goa where a leper (his disease was confirmed by a well known local medical man) betook himself far away into the jungle being driven away by his relatives and friends He lived there on caju fruits alone for months on end and was found to have rid himself of the disease This case is worth investigating

OBSERVATIONS OF TUBERCULOID SKIN LESIONS OF LEPROSY IN THE PHILIPPINES *

BY

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AND

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ONE of the recognized varieties of leprotic lesions departs widely in its histopathology from the typical, resembling instead the tissue reaction of tuberculosis. These 'tuberculoid' lesions are not without interest in connection with diagnosis, treatment, and observation and control of patients who have become bacteriologically 'negative' under treatment. As yet, however, their occurrence is perhaps looked upon rather as a matter of curiosity than of practical importance. Heretofore the condition has not been recorded from the Philippines, nor has its occurrence in patients who have become negative been reported.

The typical manifestations of leprotic localization are infiltrative rather than proliferative. Large mononuclear scavenger wandering cells make up or at least predominate in the infiltrations. They may acquire globi or become foamy, but they remain distinctly leucocytic in appearance and distribution. There is seldom important accumulation of the 'round cells' of chronic inflammation. The connective tissue increases but moderately when at all, and necrosis is ordinarily absent. In tuberculosis, on the other hand, the wandering cells probably contribute only a part of the so-called epithelioid cells, which become massed in compact foci in and about which local proliferation is usually evident. Round-cell accumulation is the rule. Caseation necrosis occurs except in unusually benign infections, and there is a decided tendency to fibrosis. So, in view of the normal blandness of the reaction to leprotic infection, the occasional production of tuberculoid lesions is of special interest, indicative of the action of special influences.

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* Published with the consent of the Director of Health upon recommendation of the Philippine Leprosy Research Board.

Tuberculoid Lesions in Diagnosis

Not many observations of tuberculoid changes in leprosy have bee
in detail Jadassohn is credited with the first report in 1898 soon fo
Klingmuller(1) We know of record of subsequent reports by Kedr
Pautrier and Boez Darier* Tschernogubow and Pawlow(3) and most
Tebbutt(4) The lesions as described appear in general as plaques with r
reddish slightly raised margins being flatter smooth perhaps slightly
or even crackling centrally Some suggest lupus though they do not ulcer
is scarring a part of the picture There are sensory disturbances in the
themselves and elsewhere and other changes of leprosy Bacilli have usual
been found either in smears or sections The condition has been related t
usually bacillus free lichens that occur in tuberculosis and to similar condi
in other infections

It is highly probable that such lesions occur more frequently tha
recognized Most of the recorded cases were observed in European clinics who
being unusual they were specially studied to establish diagnosis Reports fro
the more important endemic regions are all but lacking At the Strasbou
Conference Noel stated that the type is very common in Africa he mentione
having observed three cases Rabello said that similar lesions are seen in Brazil
and remarked on the embarrassment they cause in diagnostic work It is not
evident to what extent these statements were based on findings in sections which
are essential Tuberculoid lesions as a whole are probably not sufficiently
distinctive to be identified positively on clinical evidence alone

This being the case it need occasion no surprise if such lesions are passed over
in the examination of leper suspects at least where such suspects are no novelty
This may occur whether the purpose be to detect all cases of leprosy in which case
as by certain workers in India† diagnoses will be made primarily on clinical
grounds and negative smears make no essential difference or whether the
purpose be to select those lepers who because bacteriologically positive on
standard examination are to be segregated as in the application of regulations
such as those for the control of lepers in the Philippines In neither case need
attention be paid to the histological character of a clinically positive but bacterio-
logically negative lesion

We know of no case recognized in the Philippines by the usual diagnostic
bodies During the several years that one of us (H W W) was connected with the
official examining committee in Manila no attention was paid to the matter Only
rarely was tissue from a suspect examined and then in another connection Case I

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* For these two reports and discussions on which together show the present knowledge of the
matter see the Transactions of the Third International Leprosy Conference Strasbourg 1923 (Bailler
et Fils Paris 1924) Unless otherwise indicated the statements as to its occurrence here quoted are
from this source

† Dr E Muir has stated in personal communication that probably half of the lepers under treat
ment in his clinic had not been found bacteriologically positive



cells or a Langhan's giant-cell or two in otherwise ordinary leprosy, while on the other hand they may involve an entire lesion Such changes have usually been found in the skin though occasionally they are in nerve, testis, or lymph node The observations have not been collated They are mentioned here merely because they indicate that this change may occur during treatment, it is very probable that it does happen

Such a development would not be a matter of indifference to the patient It seems that such lesions on the whole tend to persist, epithelioid foci apparently do not resolve as readily as ordinary leprotic infiltrations Since the lesion would remain clinically ' positive ' to the eye of the physician, the patient would not be thought of as a possible candidate for the negative list He would thus remain unnecessarily long in segregation, and be (probably) unnecessarily discouraged concerning his condition To detect even the more distinctive cases will require special attention, unless perhaps periodic general bacteriological tests be made However, it is problematic whether the frequency of the contingency suggested is great enough to justify this measure as routine where large numbers of cases are being handled

Tuberculoid Lesions in Negatives

Tuberculoid lesions that develop after patients have become bacteriologically negative and are under observation preceding release are of special interest Most of the material that we have studied especially is of this nature The cases first discovered puzzled the ' Negative ' Committee because of the persistence of lesions that in colour and consistence, seemed clinically positive but which were as persistently negative for bacilli in smears The condition revealed by sections was at first thought to be low grade tuberculosis, an idea that was soon abandoned

Clinically the cases are not all alike In some, hypo pigmented macules of apparently common type took on peculiar characteristics Certain other cases stand by themselves in that multiple lesions appeared suddenly, as do the manifestations of ordinary ' lepra reaction ' Following are abstracts of two representative cases * It is to be recalled that all Culion inmates must be found bacteriologically positive before being brought to the colony

Case 1 —E M male Filipino aged 34 when admitted in October 1922 the manifestations were chiefly neural (anæsthesia, atrophies contractures) with pale macules In March 1924 he was put on the negative list Late in 1925 two pinkish, slightly elevated patches were noted on the face and neck and early in 1926 others appeared some on the face which was much pock marked Smears always negative In May lesions less elevated though still conspicuous when tissue was removed in June However, they were not distinguishable in a photograph Sections showed tuberculoid lesions The guinea pig injected was sacrificed and found negative Subsidence was rapid at least to a certain point, when paroled irregular erythema and slight infiltration persisted

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* The clinical data are available through the courtesy of the chief physician Dr C B Lara, and of the treating physicians in charge of the patients The members of the ' Negative ' Committee have interested themselves in the matter, and the biopsy material has been obtained chiefly through the co operation of one of them, Dr José Samson, in charge of surgical work

Comment —This was the first 'negative list' case to be studied The pathological diagnosis was 'tuberculosis or tuberculoid leprosy,' but we were inclined to the former diagnosis before the result of the inoculation was apparent The patient was paroled for the reason that whatever the ætiology of the lesion authority lacked for continued detention in view of the negative smears

Case 2 —D B female Filipina, aged 22 when regular treatment began in 192? only macules and anæsthes as recorded Slowly improved until declared negative in May 19?? Cheeks and ear lobes then reddish In September there was a sudden eruption of several elevated reddish macules on

nasal septum

Comment —The sudden eruptions of lesions the first accompanied by fever are specially interesting Clinically, this was an ordinary lepra reaction signalling an exacerbation of the disease or since the case was on the negative list a recurrence However bacilli did not become abundant and the lesions cleared up The second reaction was more localized than before and this time smears from the macules which proved to be tuberculoid have been persistently negative though bacilli have subsequently been found elsewhere

Discussion

It is probable that the tuberculoid lesions do not constitute a clinical entity whatever clinical features they may have in common and that their positive identification depends on laboratory study However the cases discovered

be seen

The problem of differentiating actual tuberculosis arises in every instance This *is not solely because the histological characters are those usually associated* with tuberculosis there is also the fact that in leprosy there is an unusual tendency for the localization of tuberculosis secondarily in peripheral tissues This has repeatedly been remarked on Wade(5) has recently commented on the contrast between the frequency with which tuberculosis appears in tissues for which leprosy shows special predilection especially the superficial lymph nodes and the tendency of leprosy to avoid the tissues of special predilection of tuberculosis (lungs intestines) In some of the skin lesions of our autopsy material we have seen lesions that we still believe to be tuberculous complicating or at least in close association *with leprotic lesions Lie(6) has cultivated the tubercle* bacillus from skin and lymph nodes of lepers This authority has been most doubtful of the leprotic

origin of the tuberculoid lesions As he most conservatively puts it, there are cases with 'tuberculoid' changes which cannot be proven to be due to the tubercle bacillus if there is anaesthesia they are called leprosy However, in spite of the possibilities of doubt it is the concensus of opinion that the changes are actually due to leprosy This is the more reasonable since it is becoming realized that analogous and essentially similar 'lichenoid' changes occur in other disease A unique observation of fairly direct evidence is recorded by Pautrier and Boez A piece of skin in which they had not been able to find bacilli was inoculated under the skin of a guinea pig In the purulent content of a small abscess that developed they found fairly numerous acid fast bacilli, some in intra cellular globi These they considered in all probability lepra bacilli temporarily multiplying The animal did not develop tuberculosis

We ourselves were originally inclined to ascribe these changes to tuberculous invasion and to involve in explanation a local lowering of resistance—or increase of *suitability—due to the leprotic infection The results of guinea pig inoculations* alone have been sufficient to convince us that this is not the case Granting that tuberculous tissues sometimes fail to infect the animal it is not to be believed that several pigs inoculated from as many cases would fail of infection From the clinical viewpoint the lack of progression ulceration or scar formation is decidedly against actual tuberculosis though the possibility of a complicating non bacillated 'tuberculide' might sometimes be difficult indeed to eliminate The 'reaction' onset in certain cases is strong presumptive evidence of leprotic origin, as is the finding of leprosy bacilli in the lesions, as was sometimes done

In two instances guinea pigs inoculated with skin material acquired tuberculous infection In both cases there were features that distinguished the lesions These were a tendency to ulceration necrosis *other than unimportant* 'necrobiosis' a tendency to fibrosis and fairly marked proliferation of the epidermis Changes so marked we would not call tuberculoid It may not be possible absolutely *to eliminate the possibility of tuberculosis in a given case,* but we are inclined to believe that a positive diagnosis of tuberculosis can be made in sections when this is detectable by guinea pig inoculation

Assuming that the tuberculoid reaction is due to leprosy, there arises the question of mode of production, the reason for this unusual reaction Jadassohn* thought it due to a special degree of allergy of the organism Darier points out *that similar results may obtain* not only in tuberculosis but also in other chronic or subacute infections, as syphilis, leprosy, the mycoses, etc , it is therefore not specific of any one As indicating that there is an immunity factor Rabello pointed out that in Brazil tuberculoid lesions are most common among those in whom leprosy is least frequent (the negroes) It is probably not without significance that they have invariably(?) been found in the so called

* Quoted by Darier, Transactions, Strasbourg Conference The other quotations at this point are also from this source

maculo anæsthetic cases in which resistance is highest All of our cases have been essentially of that nature though by no means bacteriologically negative

That there is an allergic element we believe is clear This is evidenced by the sudden ' reaction onset of the lesions in Case 2 Possibly antigenic material from some focus was suddenly disseminated through the blood stream The skin was unquestionably hyper sensitive presumably to the proteins of the bacillus there could not otherwise have been so much reaction to the practically atoxic leprosy bacillus even had it been present in numbers The fact is that bacilli were so scarce that they could not be found on careful search of well stained sections Indeed it may well have been that no living stainable bacilli were present at all the reaction may have been caused by dead and degenerated bacilli Another possibility exists that instead of dissemination of antigenic material to hyper sensitive tissue tissue already containing the antigenic substance may become hyper sensitive The essential feature is the same in either case

Be this as it may there arises an interesting question in connection with prognosis Is the condition responsible for this unusual reaction beneficial to the patient ? Possibly so on the whole but Case 2 and certain others indicate that in some instances it is not We would wish to see the other cases observed for a period of years to determine the effect in them As a matter of fact it is believed very much to be desired that so far as possible cases of tuberculoid leprosy be detected and be followed with special attention

Summary

Three instances of tuberculoid lesions in lepers under diagnostic examination are recorded the first from the Philippines The probability that such cases are usually overlooked is discussed

The possibility that such lesions may arise in patients under treatment is suggested and the desirability that if this does occur it be recognized is pointed out

The development of such lesions in patients who have become negative under treatment is recorded and the causation discussed The desirability of studying this condition is suggested

References

(1) Klingmüller V (19 0) *Lepra* 1 30

( ) Kedro ky W (1914) *Arch f Dermat u Syph Orig* 1) 27 (Abstracted in *Trop Dis Bull* 4 514)

(3) Tschernogubo N A and Ia l w N F (19 5) *Dermat Wochnschr* 81 1771 (Abstracted in *Trop Dis Bull* 19 6 2 16)

(4) Tebbutt (19 6) *Med Jour Austr* 2 381

( ) Wade H W (19 6) Remarks on the comparison of leprosy and tuberculosis First National Congress on Tuberculosis Manila December

(6) Darier and Lie (19 3) Transactions Third International Leprosy Conference Strasbourg

THE PRESENCE OF *MYCOBACTERIUM LEPRÆ* IN THE PLACENTA AND UMBILICAL CORD *

BY

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THE presence of *Mycobacterium lepræ* in the placenta and umbilical cord has been investigated by several observers With a few exceptions most of them examined very few cases Rodriguez(1) was the first to examine this material in this colony and the present work is actually an extension of his He reported having found the organism four times in the cord and once in the placenta in 15 specimens examined

Sugai and Monobe in their first report(2) found the organism in 9 out of 12 placentas examined later they(3) again reported having found the organism in 4 out of 12 placentas They also claim that they found the bacillus in the circulating blood of 10 out of 12 newly born children of leper parents San Juan(4) has also found acid fast bacilli in the placenta of lepers

Jeanselme(5) examined histologically the placenta and cord from a maculo anæsthetic patient and found no microscopic lesions Sandes(6) says that microscopic examination of the placenta has shown no bacilli nor lesions attributable to their previous presence Dentu(7) studied the placenta in 5 cases and found them absolutely normal

It is seen that with the exception of Rodriguez and Sugai Monobe opinions have been based on negative findings in very few cases

I have been fortunate in having opportunity to examine many placentas as in this colony marriage between lepers though discouraged cannot be prohibited and there are some 40 to 60 births a year This being of the size of an average town it was impossible to obtain the placenta in all cases or as soon after delivery as would have been preferred They were taken to the laboratory at once when delivery occurred in the hospital and in from 3 to 12 hours after delivery if it occurred outside

Technic —A portion of the cord was rinsed in tap water to free the surface from maternal blood laid on a board and an incision made longitudinally Direct smears

* Published with the consent of the Director of Health upon the recommendation of the Leprosy Research Board.

were made from the cut surface and from the cord blood Several direct smears were likewise made from deep incisions made into the placenta A second piece of cord about 15 cm long and a portion of the placenta approximately 40 grammes in weight were rinsed in water and wrapped separately in several layers of new clean gauze These were then pressed separately The press I used consists of a heavy perforated steel cylinder of about 55 mm in diameter with removable bottom and accurately fitting solid metal plunger This set in a metal tray with a spout, was subjected to heavy pressure in a hydraulic press

The material obtained was made up of bloody fluid with pulpy sediment This was transferred to clean sterile test tubes and centrifuged for a very short time to throw down the bigger particles The turbid fluid was transferred into another sterile tube and centrifuged for about 1 hour at high speed The clear supernatant fluid was thrown away and several rather thick smears made from the sediment These were then fixed by heat and stained in the usual way

Special precautions were taken to prevent contamination of the material with organisms from extraneous sources

Results —Of 104 specimens examined 57 or 53 per cent were found positive either by direct smear or by the concentration method In 25 cases or 24 per cent the organism was also found in the cord In only one case was the organism found in the cord and not in the placenta In 15 cases or one fourth of those found positive the organism was found by the concentration method only The organisms seen were of typical *M lepræ* morphology and in many cases in globus forms

In many cases that were clearly positive in direct smears or by the concentration method sections stained for bacilli were negative and in those sections found positive the organisms were few in number and required prolonged search of many slides The organism apparently showed no preference for any particular site It was found free in the blood channels in the endothelium of the blood vessels epithelium of the villi and in the connective tissue In the cord the organisms were found free in the umbilical vessels and in two instances in the mucous tissue Histologically neither placentas nor cords showed any pathological changes attributable to leprosy

Comment —That the placenta may harbour the organisms of a disease present in the mother has been well established It has been repeatedly shown both histologically and by inoculation experiments that the placenta of syphilitic mothers contain the treponema and in tuberculosis many workers have reported finding tubercle bacilli in the placenta Schmorl and Gripe(8) found tubercle bacilli in 9 out of 20 placentas examined and estimate that 50 per cent of pregnant phthisical women have tubercle bacilli in their placentas

The question as to whether the placenta acts as an efficient filter against bacteria has given rise to a great deal of discussion Certainly there are factors to be considered in this question such as the character of the maternal infection and the biological properties of the infecting micro organism (including possible transitory changes in its morphology) However that bacteria do pass the placenta and gain

the fetal circulation has been definitely shown in several diseases among which may be mentioned syphilis typhoid fever, malaria anthrax pyogenic infections and leprosy Experimentally, Sugai and Monobe(9) have shown lepra bacilli and tubercle bacilli in the blood of all fetuses 48 hours after injecting an emulsion of the corresponding organism into *pregnant guinea pigs*

In the present study in 25 cases or 21 per cent, the organism had actually passed through the placenta and was found in the cord blood Furthermore, I have seen the organism in still born fetuses and in infants born of leper parents

In tuberculosis it is rarely that newly born infants of tuberculous mothers show *distinct tuberculous changes although the presence of the bacillus has been* demonstrated with comparative frequency microscopically and by inoculation tests Schmorl and Birsch Hirnschfeld(8) found tubercle bacilli in the placenta and cord of a fetus the mother having died from acute general miliary tuberculosis in the seventh month of pregnancy, and Londe(8) in some cases obtained infection of guinea pigs *that had been inoculated with placental tissue, fetal blood and other* organs of apparently normal offsprings of tuberculous mothers This condition has been named by Honl(8) 'status bacillaris' to distinguish it from congenital tuberculosis with structural changes although the tissues in both conditions are capable of infecting guinea pigs This same condition has been reported in typhoid and malaria

The existence of an active disease in the fetus is however, an entirely different question and two other factors will have to be considered These are the susceptibility of the fetal tissues and the amount of anti bodies or more probably anti bacterial ferments present in the placenta

Most of the discussion on transplacental *transmission of disease in man centers* around tuberculosis but at present intra uterine infection, although rare, is considered established by a number of well authenticated cases on record In these cases the disease developed in children born of tuberculous mothers in so short a time and under conditions that precluded post natal infection Holt(10) Kuhle(11) Moll(12) and others have reported clear cases of *congenital tuber culosis*

In leprosy, where there is a tremendous infection and in which bacteremia occurs particularly during lepra reaction it is to be expected, and as it has been found, that the placenta should in a number of cases be also infected That the organism *enters the fetal circulation in a considerable proportion of cases* has also been shown

As to what finally happens once the organism has gained the fetal tissues we can but speculate Certainly there is the possibility, as is the belief of Baum garten(8) in tuberculosis, that the organism may remain dormant for a long period of years to flare up by intense multiplication when *for some reason* the natural resistance of the body fails Against this view, we have the fact, as it has been shown in Hawaii(13) and in India(14) that only a very negligible per cent of children of lepers when removed soon after birth acquire the disease

We are therefore forced to the conclusion that the organisms, on reaching the fetal tissues in the large majority of cases, stay dormant for some time and are finally destroyed The possibility however, of the infection occurring during the intra uterine life of the fetus should be borne in mind, particularly where the disease manifests itself in early infancy such as in the case reported by Goodhue(15) in which the infant was segregated within a few hours after birth and developed the disease 18 months later by Makayo(16) in which the infant 3 months old was found bacteriologically positive and with definite leprotic infiltration of the skin, and by Rodriguez(1) where there were suspicious lesions in six Culion children between the ages of 3 and 6 months, and in 3 of them these same lesions became bacteriologically positive from 1 to 1½ years later

SUMMARY AND CONCLUSION

Of 101 placentas examined, 57 or 53 per cent were found positive In 25 cases or 24 per cent the organism was also found in the cord blood In only one case was the organism found in the cord and not in the placenta Histological examination of placentas and cord showed no pathological changes attributable to leprosy

The bacillus of leprosy reaches the fetus in a considerable proportion of cases, although in the large majority it is probable that they are finally overcome

Intra uterine infection in leprosy should be considered in some cases particularly when the disease develops in early infancy

REFERENCES

(1) RODRIGUEZ (1926) *Phil Jour Sci*, 31 No 2
(2) SUGAI and MONOBE (1913) *Tokyo Med Assoc*, 27, 8 (Abstracted in *Trop Dis Bull*, 2, 6)
(3) *Idem* Abstracted in *Trop Dis Bull* 1 No 10 pp 559 560
(4) SAN JUAN (1919) *Rev Argentina de Obst y Ginecol* (Abstracted in *Rev de Med y Farm*, Manila 10, 439
(5) JEANSELME (1910) *Bull Soc Path Exot* 3 pp 326—28
(6) SANDES (1911) *Brit Med Jour*, 2, 469
(7) DENEY (1910) *Bull Soc Path Exot* 3, 6 pp 412—16
(8) SCHMORL, and CRIPE (1924) Cited by Fishberg, M Pulmonary tuberculosis, Lea and Febiger Phila 3rd Ed. p 108
(9) SUGAI and MONOBE (1912) *Centralbl f Bakt* 67 336 (Abstracted in *Trop Dis Bull*, 1913, 1,553)
(10) HOLT L E (1916) Diseases of Infancy and Childhood Appleton & Co., N Y
(11) KUBLE (1924) *Deutsche Med Woch* June 13, 1 796
(12) MOLL (1924) *Monatschr f Kinderh*, 28, 58 (Abstracted in *Amer Rev Tuber* 1925 11, 81)
(13) HASSELTINE *U S Pub Health Bull* p 141
(14) JACKSON J (1910) "Leprosy" Marshall Bros Ltd, London, Revd Ed
(15) GOODHUE *Pub Health Bull* No 75
(16) MAKAYO *Jour Cut Dis*, 33, 7,653

TUBERCULOSIS.

INCIDENCE AND TYPES OF TUBERCULOSIS MET WITH IN BENGAL

BY

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Epidemiology

Tuberculosis was much rarer in rural areas in Bengal 50 years ago, i.e., so far as the memory of the medical men of the present generation goes. It has considerably increased within this period due to several factors, among others:—

(a) Lack of calcium containing food, fats, milk and fruits in the dietary of the people, thus supplying a devitalized soil for disease incidence,

(b) Rapid transport facilities favouring diffusion,

(c) Industrialization of rural areas and urbanization,

(d) General ignorance of health matters and lack of sanitary sense in the people, of which promiscuous spitting, eating and drinking from the same vessel and sleeping in the same room are the most important.

It is estimated that in Calcutta alone there are over 20,000 cases of 'open' pulmonary tuberculosis and that there are about 200,000 such cases (or a little less than 0.5 per cent of the population) in Bengal.

Over 900,000 people die of 'fevers' in Bengal every year. It is estimated that about 10 per cent of them (or 90,000) are really due to tuberculosis. For every death of tuberculosis there must be at least seven others suffering from it, i.e., there are at least 630,000 persons in Bengal suffering from it at any given time. *Approximately 2 per cent of all cases attending the polyclinic of the Medical* College Hospitals in Calcutta show some form of tuberculosis.

A comparison with the mortality statistics of other countries will be apparent from the following table:—

Table I

	Calcutta	France	Great Britain	U. S. A.
l deaths	35 per thousand	17.76 per thousand		
erculosis	2.4	2.13 ,,		
ionary tuber losis	2.3 ,,	1.77 ,,	0.96 per thousand	1.28 per thousand

Degree of Tuberculization

Let us now get an idea about the diffusion of the disease in Bengal We know that an approximate idea about the degree of tuberculization of a people can be obtained from *cuti reaction* results Our enquiry, which is still going on has been limited for the purpose of this report to 3,075 cases in rural, urban and industrial areas The results will be apparent from a glance at the tables and charts given below In doing the test von Pirquet's technique was followed, using pure tuberculine Brute, prepared at the Pasteur Institute, Paris Reactions were recorded as positive after an interval of 48 hours if there were redness and palpable (between two fingers) œdema around the scarified area The intensity of the reaction was noted under four heads—strong (indicated by +++) when the diameter equalled or exceeded 1 cm, moderate (indicated by ++), when the diameter was between 0 5 to 1 cm, weak (indicated by +) and, when the diameter was below 0 5 cm, doubtful (indicated by ±) In estimating the number of total positives, half the number of doubtful cases was included

Table II.

According to Age and Intensity of Reaction

A *Jail Cases*

Age	Intensity of reaction					Total number tested	Percentage positive
	+++	++	+	±	−		
16—20 years	3	32	42	19	73	169	51 1
21—25 "	16	47	88	17	103	271	58 8
26—30 "	28	64	102	57	83	334	66 6
31—40 "	8	58	121	55	100	342	62 7
41—50 "	4	23	51	14	38	130	65 3
51—60 "	5	7	25	14	13	64	68 7
61—70 "		1	4	1	8	14	Number too small for percentage
Above 70		1	4		2	7	
Total	64	233	437	177	420	1,331	61 7

B Students

Age	Intensity of reaction +++	++	+	±	−	Total number tested	Percentage positive
6—10 years		13	21	18	96	148	29 0
11—15 „		12	35	16	176	239	23 0
16—20 „	*6	21	66	14	247	384	29 9
21—25 „	1	9	37	37	90	174	37 6
26—30 „		16	8	20	38	82	41 4
Total	7	71	167	135	647	1 027	30 3

* Four of these have been in contact with pulmonary tuberculosis cases in the family

Remarks —Of the above number 24 cases have been traced to have been in contact with phthisis cases in the family six of them giving ++ reactions and the rest + reaction

C Females (chiefly students and all Hindus)

Age	Intensity of reaction +++	++	+	±	−	Total number tested	Percentage positive
11—15 years		6	4	10	24	44	31 0
16—20 „		2	8	4	6	20	60 0
21—30 „		6	4	8	6	24	58 3
31—50 „	8	12	8	16	8	52	69 2
Total	8	26	24	38	44	140	68 5

D *Infants and Children under 6 years*

Age	Intensity of reaction					Total number tested	Percentage positive
	+++	++	+	±	−		
0—5 years	1	11	7		208	227	8 4

Remarks —Of these pos tives two were cases of Pott s disease and ten were found to be in contact with 'open' tuberculous relatives (mother in 6 cases father in 2 cases, maternal uncle in 1 case)

It will be seen that the maximum tuberculization occurs between the ages of 25 to 30 years The rarity of evidence of bacillization in infants and children and the more extensive bacillization in females are noteworthy This is contrary to what one finds in Europe *The percentage of positive von Pirquet over all ages comes up to* 47 8 *per cent*

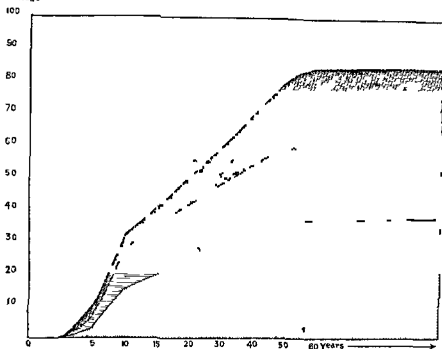
The influence of habitation in sparsely populated rural and in thickly populated urban areas will be shown by the following table We have included under the section 'rural' those who live in villages and those who have been in towns for not more than six months, 'rural urban' those who have lived in towns from six months to three years [for we have found that rural people begin to give positive reactions only after a stay of two to three years in big towns (Ukil, 1927)] and 'urban' those who have been born and brought up in towns or those who have lived there for over three years

TABLE III

Cuti reaction according to Habitation

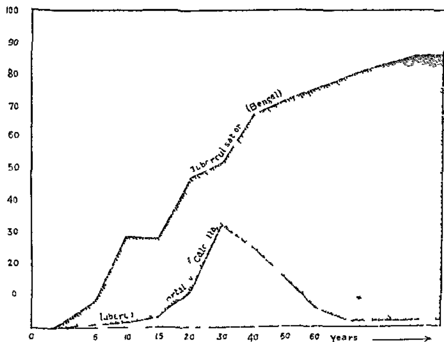
Habitation	PRISONERS	STUDENTS	FEMALES	INFANTS AND CHILDREN
	Percentage positive	Percentage positive	Percentage positive	Percentage positive
Rural	32 1	21 8	55 0	3 5
Rural Urban	50·4	37 1	78 0	5 0
Urban	74 1	32 3	86 0	9 2

CHART I



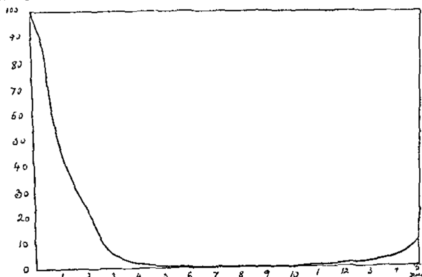
Showing tuberculization according to habitation and age in Bengal

CHART II



Showing relation of tuberculosis mortality to diffusion of tuberculosis in Bengal

CHART III



Showing age incidence and mortality from tuberculosis in Vienna (1911) (After Escherich)

The reaction according to *professions* is shown below It will be seen that carters goldsmiths and mill hands show a higher incidence than other classes School teachers students and clerks form the major portion of sanatoria cases

TABLE IV

ACCORDING TO OCCUPATION

Jail Cases

	Occupation												Total
	Carters	Goldsmiths watch makers etc	Mill hands	Ja l l f	Beggars	Tailors	Menials	Clerks school masters etc	Landholders	Shopkeepers	Cultivators		
Total number tested	43	8	70	188	5	17	346	41	10	[illegible]01	40[illegible]		1 331
Percentage positive	91 7	81 0	71 1	70 0	70 0	67 6	67 0	65 8	6[illegible] 0	64 1	43 6		61 7

TABLE V

ACCORDING TO AGE AND INTENSITY OF REACTION

Mill Hands

Age	Intensity of reaction					Total number tested	Percentage positive
	+++	++	+	±	−		
16—20 years		6	[illegible]	4	19	51	58 0
21—25	1	11	[illegible]	6	18	61	65 5
26—30		14	4	4	10	5[illegible]	[illegible]6 0
31—40		7	[illegible]	6	10	[illegible]	74 5
41—50		7	13	2	4	2[illegible]	8 0
51—60		1	5	1	1	8	81 0
TOTAL	1	46	118	[illegible]	6	[illegible]	70 3

While trying to follow the *relationship between physical build and cuti reaction* we found that the incidence was decidedly greater in people of weaker physique While trying to note the relationship between the *different communities* (Hindus Mohammedans Indian Christians and Anglo Indians) and cuti reaction we found that the Hindus and Mohammedans were almost equally bacillized and that the incidence among the Christians and Anglo Indians was 10 per cent higher

An attempt was also made to determine the *gland incidence* by palpating the neck glands Thus out of a total of 887 cases tested 284 or 32 1 per cent showed *palpable neck glands only 74 or 26 2 per cent of whom gave a positive von* Pirquet The glandular enlargement in the majority of cases therefore must be accounted for by other conditions in mouth and naso pharynx

The significance of the intensity of a positive reaction —The intensity of reaction indicates the strength of the allergic state or immunological response of the body—the stronger the reaction the better the response On a reference to the tables it will be found that out of 1 614 persons in civil life tested only 17 or 1 03 per cent showed a strongly positive (+++) reaction 151 or 9 per cent showed moderately positive (++) and the rest mild reactions Most of the cases giving +++ reactions were traced to a tubercular focus in the family It was more difficult to trace the other cases It may be that they represent a state of progressive immunity from small and repeated doses But in infants below five years we

have shown more than once that a negative cuti reaction is the rule even in highly contaminated urban areas like Calcutta A moderate or a markedly positive reaction in them points to massive infection in the family Individuals from rural areas when they showed a positive test usually gave a 'weak' reaction

The interpretation of a negative reaction —A cuti reaction may be negative due to four causes —

(1) That the dose of tuberculin has been too small to wake up a reaction,
(2) That the reaction has been done during the ante allergic (or incubation) period,
(3) That the individual is non immunized
(4) That there is no immunological response owing to its having been spent up in rapidly developing and advanced cases

TABLE VI

Comparative data in other Asiatic countries

Age	Bengal percentage positive	Cochin China (Lalung Bonnaire) percentage positive	Indo China (Noel Barnard) percentage positive	Java (De Langen) percentage positive	France (Marfan) percentage positive
At 10 years	29	35 to 40			63 7
At 15 ,,	29	64 to 75			81 9
At 20 ,,	49 7	76 to 89			89 0
TOTAL	48 0	67 0	65 0	65 0	

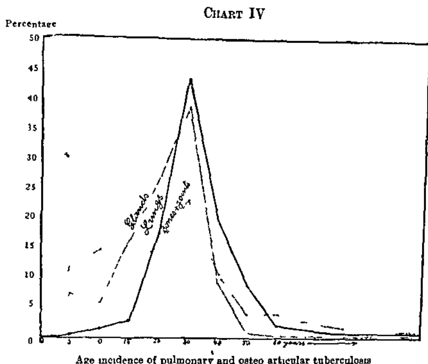
The low figure of average total positives is due to the extremely low incidence of positive cuti reaction in children

PRINCIPAL FORMS OF TUBERCULOSIS MET WITH IN BENGAL

In a series of 1,019 tuberculosis cases out of a total of 52,550 cases attending the polyclinic of the Medical College Hospitals in Calcutta the incidence of lung tuberculosis was found to be 62 8 per cent, that of glandular tuberculosis, 17 per cent, bone and joint tuberculosis 13 9 per cent, tubercular enteritis 2 8 per cent, tabes mesenterica, 1 5 per cent and other localizations formed the remaining 2 per cent Skin tuberculosis is extremely rare, being only two in the above series Out of 2,480 cases in his skin clinic Acton noticed only 18 cases of tuberculides The commonest site of bone and joint localization was found to be in the hip, next comes the spine Dactylitis of the upper extremity is commoner than any other

variety of bone tuberculosis The same incidence was noticed by us previousl in a series of 81 cases among 1,700 surgical indoor patients (Ukil, 1927) Th age incidence of the different forms of tuberculosis will be apparent from a glanc at the following chart —

CHART IV



Age incidence of pulmonary and osteo articular tuberculosis

It will be seen that between the ages of 1 to 10 years, glandular and bone tuber culosis are the chief forms, of which again bone and joint tuberculosis has a higher incidence From 10 to 15 years, lung tuberculosis occurs a little more frequently, but it is much less common than the other two forms, whose curves steadily rise till they reach their maximum at the age of 30 years after which there is a sharp decline It will be seen that lung tuberculosis also follows a parallel curve from 15 years onwards, that of females rising and falling earlier than in males The mortality from lung tuberculosis also follows the same lines, as is evidenced by mortality statistics and post mortem data The same age incidence is also illustrated by the admission registers of the different sanatoria in India

But when it comes to gland and bone tuberculosis, the picture changes, for 95 per cent of the mortality from tuberculosis is formed by pulmonary tuber culosis Primary intestinal tuberculosis and tabes mesenterica occur between the ages of 25 to 35 years We have tried to follow the evolution of glandular tuber-culosis by X rays and by cultures and animal inoculations The localization in children below 10 years is almost entirely limited to the cervical region Even in

cases of repeated massive contaminations from a tuberculous father or mother or grandparents we have failed to find any hilus involvement in the children so far observed The glandular incidence rapidly rises from this age up to 30 years the localizations being mostly in the cervical groups but also in the axillary and more rarely in the inguinal regions From 10 years onwards we find evidences of hilar involvement in many of the cases but in many others also there is no such sign in spite of greatly enlarged and caseated neck glands that is to say they remain limited to the neck glands for a considerable time in spite of such patients getting an evening rise of temperature and losing weight In contrast to the fact that pulmonary tuberculosis is practically the only lethal form of tuberculosis the glandular varieties have a very chronic course Death occurs in them usually from meningeal involvement In those cases where there are extensions to hilar glands there is also evidence of a chronic fight until ultimately extensions occur to lung areas in those who cannot put up a good fight Evidence of chronic involvement of the whole lymphatic (glandular) system is seen in some cases usually followed by lung involvement later on

Clinical types of lung tuberculosis —We have been able to tabulate the history and physical signs of 440 cases of lung tuberculosis All evidences point to a great diffusion of the disease in rural areas and of the chances of massive infection—in fact it is the only mode of infection in rural areas Contacts can be traced in most cases being transmitted by the mother father grandfather sisters brothers and wife in order of frequency

Mode of onset with hæmoptysis 68 per cent	Single hæmoptysis	23 per cent
	Recurrent	45
cough and fever but no hæmoptysis		20
slow evening fever		8
signs of active pleurisy		2 (?)
dyspepsia		2
hoarseness of voice		9 ,
asthma (above 40 years)		3 cases
Onset with pneumonia and broncho pneumonia		7
Onset like typhoid fever		3 cases below 20 years

Site of lesion

Upper lobe	Right	156
	Left	134
Both apices		27
All over lung		52
Signs of localization found in a single area		210
multiple areas		159
No lung signs detected		71
Concurrent extra pulmonary localizations in glands and bones		4

The usual signs obtained on auscultation are clicks and crepitations and frequently over more than one area in the lungs Of the very few cases observed in

children between 5 to 10 years a history of pneumonia or broncho pneumonia has been obtained followed by a persisting cough and even hæmoptysis The average duration of life in such cases has been found to be between 1 to 2 years after the onset of fever

Radiographic picture—In about 80 per cent of cases between the ages of 16 to 25 years the picture represents the usual one of hilus tuberculosis in the adult i e enlargement of root glands with fanwise peripheral extensions along peri bronchial and septal lymphatics The picture of the infantile or glandulo pulmonary type is extremely rare even in children nursed by tuberculous parents It has been pointed out that the cervical group is chiefly involved in them But our knowledge of such cases are still very limited

The calcification of the first rib in adults is very fragmentary and not uniform

Average duration of life—The duration of life depends on the dose of infection and the age It also depends on the extent and multiplicity of lesions as also on secondary bacterial associations as will be shown later The duration of life is much shorter in rural people than in inhabitants of thickly populated cities It is distinctly shorter in females It is between 6 months to 2 years in persons from 16 to 25 years of age 1 to 3 years or more in persons from 26 to 40 years of age 3 to 5 to 10 years as age advances in persons above 40 years of age

Pregnancy and lactation diabetes influenza and kala azar have been found to shorten the course

Post mortem evidence—(Based on 1 000 consecutive post mortems performed in Calcutta during the last 13 years)

The total number of tuberculosis cases in this series was 176 of which 120 died of tuberculosis of the lungs and in 56 of which death occurred from other diseases tubercular lesions were found

General summary—Deaths were found to be due to tuberculosis in 12 8 per cent of medical cases autopsied 4 8 per cent were found to complicate other diseases thus making a total of 17 6 per cent in which well marked tubercular lesions were found Primary intestinal ulceration was found in 5 1 per cent of these cases Secondary intestinal ulceration was found in 51 1 per cent of cases

Pleural adhesions were found to be very frequent multiple and extensive In 72 per cent of cases old adhesions were present and in only 12 per cent soft or recent adhesions noticed

Evidence of calcification or fibrosis of old lesions was found in 31 55 per cent of cases.

Enlarged bronchial glands usually varying in size from an almond to a walnut, were present in most of the fibro caseous types of lung tuberculosis Hilus glands of all the groups were usually involved including the broncho pulmonary glands in many cases In 26 1 per cent of cases well marked caseation was noticed, with little attempt at fibrosis

Pulmonary lesions —The main sites of lesion in the lungs usually in the form of cavities were distributed as follows —Upper lobe 47 7 per cent lower lobe, 29 per cent middle lobe of right lung 23 3 per cent There were often multiple cavities in one or both lungs 14 per cent

Broncho pneumonia with great enlargement of hilus glands as seen in the infant is a comparatively rare picture having been observed in a few adolescents between 15 and 20 years of age for there are no opportunities for systematic autopsies on children in Calcutta The prevailing type of lung tuberculosis is the fibro caseous form with primary localization in one or more of the lobes and then rapidly involving other parts Cavity formation takes place quickly in the involved lung areas In a majority of cases between 16 and 30 years the cavities show an attempt at localization but the proliferative efforts seem to be fragmentary and the barriers soon break away extending to other parts of the lung by direct lymphogenous extension showing extensive involvement over both lungs in a large percentage of cases (over 62 per cent) until ultimately the last barrier gives way to miliary tuberculosis (in 42 per cent of cases) The extensions may manifest themselves as areas of consolidation (in 34 per cent of cases) or broncho-pneumonia with exudative changes inside the alveoli (in 10 per cent of cases)

Tubercular lesions in other organs

Organ		Cases	
Intestinal ulceration with involvement of mesenteric glands		90	
Enlargement of mesenteric glands without visible intestinal ulcers		20	
Intestinal ulceration without visible lung lesions		9	
Spleen		29	
Liver		30	
General peritoneum (miliary)		23	
Kidneys	Right	17	
	Left	13	
Appendix		7	
Gall bladder		2	
Pancreas		3	
Prostate		1	
Mouth and pharynx		3	(tonsil 1, tongue 1 pharynx 1)
Larynx		15	
Trachea		3	
Pericardium		8	
Heart (right auricle)		1	
Base of brain		8	

Thyroid ..	1	
Tubercular glands other than bronchial	21	(abdominal retroperitoneal, 5, inguinal, 3, axillary, 3, cervical, 10)

General and meningeal tuberculosis in children under 10 years has been found by Rogers to be 6 7 per cent as compared with 62 7 per cent in London

Pathology of lung tuberculosis in Bengal —We have not yet been able to explain all the phenomena of tuberculous processes in this country, but what we state here to day will probably be found to be essentially true in its outline and to hold good in other parts of India

The first thing which strikes one is the comparatively low morbidity as well as mortality in childhood up to 10 years, in very marked contrast to facts in Europe (*vide* Chart III) The only forms which occur with any frequency during this period, viz, the glandular and the bone and joint forms, are characterized by well marked chronicity, and often by apparent recovery, especially in glandular tuberculosis by the time youth is reached In the case of cervical glands the infection is often limited to this group without involvement of the hilus or other groups Death from glandular and osteo articular tuberculosis at this age takes place often from meningitis without any lung lesion presumably from endogenous infection This low incidence of the various forms of tuberculosis in infancy and childhood, in presence of a low degree of bacillization at this period has been a puzzling phenomenon to us

Infections are ordinarily massive from contact cases in the family or outside and are almost entirely limited to the house The chances of contracting tuber culosis through inhalation outside the house is very limited, because of the ex tremely hot and chemically active rays of the sun in the tropics (Ukil, 1927) The extremely careless method of living in India makes the chances of chronic vaccination through inhalation or ingestion of attenuated bacilli very small

In spite of these facts, it is astonishing to see how infants and children, nursed by tuberculous parents, regularly gain weight, though invariably affected with enlarged cervical glands We have seen a few guinea pigs inoculated with tuberculous material regularly put on weight while showing at autopsy extensive tubercular lesions Exactly where and how the barrier breaks down it is difficult to say We have also noticed that the more such children live out of doors during the day, the longer and better do they resist the onslaughts

The apparent immunity(?) in childhood disappears as soon as the age period steps beyond 15 years Between this period and the 40th year we find the different forms of tuberculosis in the largest numbers What constitutes this breakdown of barriers is still under study But the pathological anatomy as well as radiographic evidences and the results of the cuti reaction all point to the changes being due to a partially immunized soil being invaded by massive infection The immunity of the well immunized individual or the immunity developed by minute doses

received at infrequent intervals we see only in individuals above 40 years and in thickly populated urban areas

The explanation of massive infection on an imperfectly immunized soil answers many of the points The heavy chronic involvement of the lymphatic glands with frequent caseation and liquefaction and enlargement and caseation of bronchial glands as well as the fibro caseous and consolidative changes over multiple areas in the lungs with little attempt at repair in young individuals support this opinion Another fact in support of massive infection is the comparative frequency of primary intestinal tuberculosis presumably from swallowing heavily contaminated food or drink The comparative frequency (about double that in Europe) of a caseous involvement of and limitation to glands of the cervical group points to the frequency of infection through mouth and pharynx

After the lungs are once involved the course of the disease is much shorter here than in Europe The more acute course in females is probably due to their close and sedentary life and to early marriage and child bearing The evolution of the form seems to depend more on the dose of infection (massive infection) here than on the imperfect immunization of the individual Only 30 per cent of the sputa of suspected tuberculosis cases show tubercle bacilli by the staining methods

While trying to find out whether there are any other explanations for the more acute course of lung tuberculosis in this hot and humid country besides massive infection and imperfect immunization it struck us that the *secondary bacterial flora* in open lesions of lung tuberculosis might play a part in accelerating the degenerative processes in the tropics From the cases so far studied we have found that secondary bacterial associations are present in over 80 per cent of open or tubercle bacilli positive cases i e about double that in Europe (*cf* Benzaçon Thue and Ehrardt) Of the secondary organisms thus far studied the following *ærobic* bacteria have been noticed in order of frequency streptococcus staphylococcus Gram positive diplococci diphtheroid bacilli and yeast cells The *anærobic* bacterial flora thus far studied have frequently yielded two varieties viz streptococcus anærobic and some varieties of Gram positive cocci in clumps They have been found to be definitely pathogenic for small laboratory animals the lesions ranging from inflammatory swellings of greater or less intensity sometimes followed by abscess formation and more rarely by death Fuso spirochætal association was present in some cases

As regards the question of re infections in the evolution of tuberculosis here it seems that the endogenous process is quite a common method in comparison with the exogenous

BACTERIOLOGICAL TYPES

It is well known that cattle in India are rarely infected with tuberculosis and it has been shown by Soparkar (1926) that they are more resistant to tubercular infection than European breeds in spite of their poor physique Milk has also been found to be free from tubercle bacilli by animal inoculation with several

hundreds of samples (Joshi, 1911) Tuberculosis of glands, bones and joints has once been shown by Liston and Soparkar (1917), in Western India, to be due to human tubercle bacilli The question has again been taken up by us in Eastern India and up to the time of writing the paper the rabbits inoculated even with 1 milligram of tubercle bacilli intravenously with 10 strains have not died within two and a half months

PREVENTION OF TUBERCULOSIS IN INDIA.

BY

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TUBERCULOSIS has been a prevalent disease in India especially in cities from very ancient times But it has assumed serious proportions, since the introduction of rapid transport facilities urbanization and industrialization of rural areas These necessary concomitants of modern civilization have disturbed the socio economic fabric of the country to such an extent that the people have not been sufficiently able to re adjust their habits and life to altered environment by increasing the income *per capita* and by ensuring a proper supply and transport of food materials, with the result that there is a lack of calcium containing food fats milk and fruits in the present dietary of the people The minimum requirements of a healthy dietary for an Indian cost 6 annas or 6*d* , a day whereas his average daily income is only one third of this With this diet he may be said to exist but not to live. A majority of them have not the will to live properly and to have an adequate and rational diet and clean surroundings This must be inculcated into their minds, if the preventive campaign against tuberculosis in India is to be made effective

Among other causes may be mentioned, defective school hygiene (in some sanatoria students and teachers form about 40 per cent of the annual admissions), high incidence of other devitalizing diseases such as malaria, kala azar, pneumonia, influenza, puerperal diarrhœa and dyspepsia , and defective house construction in cities favouring ' suffocation behind the purdah ' (to quote the former Health Officer of Calcutta) But the most dangerous of causes is general ignorance in health matters and lack of sanitary sense of the people of which promiscuous spitting and eating or drinking from the same vessel are the most important This habit is one of the chief causes of the spread of massive infection from man to man in houses, bureaux, schools, boarding houses, military barracks, coolie lines , in fact, wherever there is a large agglomeration of people Diffusion takes place entirely *under the roof* (i e , in the shade) from man to man, for it has been shown (Soparkar) that expectorated sputum gets dried up and the bacilli killed in five to six hours under the direct rays of the sun So the infection of vaccination by attenuated bacilli is less common here than massive infection Non immunized or imperfectly

immunized people come to the endemic areas in towns or industrial areas in quest of livelihood or wealth, get the infection usually by massive infection from other cases go back to their families in villages and create new foci of disease The custom of living in a joint family stands at a disadvantage here, due to ignorance in health matters Many of these cases come to towns and stay in various houses for treatment, which are never disinfected We have shown that a stay of two to three years in urban areas is necessary for non immunized people to give a positive von Pirquet reaction

We have shown in another paper that there is an average bacillization of 50 per cent of people at the age of 20 years, a number far too short of European countries The number of imperfectly immunized individuals is much more than either the well immunized type in thickly populated towns or the non immunized in the interior of the country away from railways or transport routes

Practically 95 per cent of the deaths in tuberculosis are caused by the pulmonary variety Tubercle bacilli reach the exterior by sputum fæces (in over 50 per cent of the cases with lung lesions) urine (in 10 per cent of cases) suppurated lymphatic glands and osteo articular lesions

It will not be an over estimate to state that there are a million and a half of 'open' phthisis cases in India at the present moment The number of incipient tuberculosis cases and of the pre tuberculous children and adolescents must be a legion The magnitude of the problem of any campaign of prevention in India will be realized when we know that the chief source of infection is massive infection from the bacillus carriers and the chief difficulty to be surmounted will be the removal of the colossal ignorance of the general population in health matters

Thus, any scheme of combating tuberculosis must consist of —

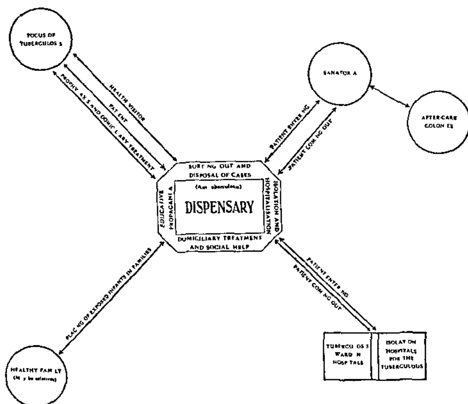
(1) *directly* attacking the contagion by early diagnosis and spotting out the diseased, their isolation in special hospitals and sanatoria and their after care, domiciliary treatment and education of the tuberculous patient, isolation of the predisposed, protection of the exposed and preservation of infants and non immunized by vaccination, isolation and other methods, and

(2) *indirect* methods for removing factors which favour contagion, e g, raising the standard of living as regards an adequate supply of suitable food, raising the hygienic standard of the home by ensuring cleanliness plentiful supply of fresh air and sunlight and giving up unhealthy habits, such as promiscuous spitting and faulty disposal of excrement and infective matter, improvement of general hygiene and of habitation in cities, segregation and supervision of barracks or coolie lines in tea, colliery and industrial areas, amplifying the laws for the notification of diseases, modifying the

Factories Act to provide for compulsory health insurance and enacting laws for the compulsory health insurance of clerks, menials, school teachers and other classes of workers; and finally co-ordination between the different anti tuberculosis organizations.

The direct methods may be graphically represented by a diagram (partly after Leon Barnard) as below —

DIAGRAM.



It will thus be seen that the anti tuberculosis dispensary is the basic organization for spotting out cases, as well as for their disposal, to appropriate places (sanatoria or hospitals), for educating the patients and giving relief to them and also for general anti-tuberculosis education

The work of the best sanatoria in India shows that sanatorium treatment here yields equally good results with other countries Ten years' working of the

Madanapalle sanatorium shows that the disease was arrested in 86 per cent of patients in stage I and 36 per cent in stage II of the Turban Gierhardt nomenclature Tubercle bacilli disappeared from the sputum in 40 per cent of cases Fifty four per cent of patients were found to be living and earning their livelihood up to five years after discharge Sanatorium treatment will be found to be of great value not only in curing or improving a case, but also as a place for the education of the patient and for the control of the output of bacilli

Sanatorium treatment, to be effective, must be made available to the poorer sections of the people Among patients running to the existing sanatoria students, teachers and clerks form the major portion, next come the cultivators and labourers There are about a dozen sanatoria in India with a little over 400 beds to meet the requirements of a million and a half of 'bacillus carriers' There are also very few isolation hospitals and anti tuberculosis dispensaries There are no seaside sanatoria for non pulmonary cases yet There is also no co ordination between the different organizations Very often advanced cases are sent to the seaside and high altitude sanatoria for treatment, without regard to consequences There is a good deal of ignorance among medical men as regards selection of cases for climatic sanatoria at different altitudes, location and climate An altitude of 3,000 feet above sea level has been found to yield the best results for the majority of cases This emphasizes the need for special training of medical men in the early diagnosis and treatment of tuberculosis cases, the prevailing types of which we have described in another paper

Of factors which are unfavourable for sanatorium treatment, excessive heat and humidity have been found to be two of the most important The selection of a site for a sanatorium is of great importance in the success of an institution As regards cases suitable for artificial pneumothorax, only 25 per cent have been found to have yielded good collapse of the lung owing to frequent extensive pleural adhesions found in lung tuberculosis in India Frequent bilateral and multiple lung involvement must also be borne in mind in selecting a case for artificial pneumothorax As regards helio therapy, direct sun's rays have been found to be injurious to the majority of tuberculosis cases, but beneficial results have been observed with rays filtered through the shade of a tree No scientific work on this subject has, however, been done here yet

As regards the after care of patients discharged from sanatoria, tuberculosis colonies can be ideally formed in a country where nature has compelled the people to live an open air life Gardening and light agriculture silk farming and weaving, poultry farming card board box manufacture, book binding and knitting are some of the occupations to which they may be trained and employed with profit

As regards the possibility of employing the B C G vaccine of Calmette for the protection of the non immunized, we tested, according to the instructions of Professor Calmette, 220 individuals at different ages in different environments by two consecutive von Pirquet at 8 days' interval to see how far they show Koch's

phenomenon of hypersensibility to infection The results are shown in the following table :—

Age groups	Total tested	Total positive	Percentage positive
0— 6 years	45	2	4 4
6—10 „	*Nil*	*Nil*	*Nil*
11—15 „	*Nil*	*Nil*	*Nil*
16—20 „	21	5	23 8
21—25 „	41	6	14 6
26—30 „	31	6	19 3
31—35 „	30	11	36 6
36—40 „	16	5 5	34 3
41—50 „	25	9	36 0
51—60 „	8	2	25 0
61—70 „	2	1	50 0
TOTAL	219	47 5	21 5

Thus, besides newly born infants in a tuberculous environment children and adults who may be exposed to infection and who show a negative cuti reaction twice when done at an interval of 8 days, delicate people, especially those with impaired digestion and bad physique who are found suitable by the above test, when they come to live in big towns, and military recruits from villages and gangs of labourers or servants from rural and uninfected regions when they are brought to big towns or industrial areas or where there is a large agglomeration of people will be found suitable for being protected with inoculation of B C G vaccine

As regards the innocuousness of the B C G strain, we did a number of experiments by inoculating *subcutaneously* doses of 5 milligrams 20 milligrams and 100 milligrams to guinea pigs and *intravenously* doses of 30 milligrams, 60 milligrams and 100 milligrams to rabbits The experiments so far conducted have agreed with the findings of Calmette Remlinger and Bailly, and of the Ukrainian Commission We have had the strain in our laboratory for a year without any evidence of a return to virulence The strain thus appears to be a stable and non virulent one

Protection experiments done in France North Africa Belgium, Rumania, Greece, Russia and Indo China have so far shown good results In France the mortality of 25 per cent in infants reared in a tuberculous environment within the first year of life has been brought down to less than 1 per cent by the use of this vaccine Carefully watched experiments with proper controls ought to be done in

other countries on individuals as above indicated The organization of such a service is especially important for countries with a low degree of bacillization and for general use in uninfected infants in a tuberculous environment Oral or sub cutaneous inoculation of this vaccine in man has not so far given rise to any accidents From all the evidence it appears that its use is likely to reduce the morbidity and mortality of a large number of non immunized infants, children and adults in Asiatic countries who are likely to be exposed to massive infection If the experiments succeed, it may form one of the strongest agents in our anti tuberculosis armamentorium

The problem of tuberculosis has now assumed an international aspect and is very important to India with her land and maritime relations with other countries Asiatic countries are still much less bacillized than those of Europe or America As a cause of morbidity and mortality, it is one of the most important of diseases The incidence and toll of leprosy in India is much less in comparison with those of this socio economic disease Yet its claims have not attracted the measure of attention it deserves from medical men as well as the State It will be to the interest of all the countries concerned to co ordinate their efforts in the anti tuberculosis campaign

TUBERCULO REACTION DE VERNES A LA RESORCINE

PAR

MARCEL LEGER

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Bien que la tuberculose soit une maladie éminemment contagieuse elle est, en principe, évitable, puis que l'on connait le germe pathogene specifique et les modes de transport de ce germe du sujet malade aux sujets sains La contagion se produit surtout parce que le diagnostic n'est porté que tardivement

Il faudrait depister, dès le début, les tuberculeux on les empêcherait ainsi d'être des semeurs de bacilles, et on les mettrait dans les conditions les meilleures pour resister au mal et guérir Comme l'ecrivait en 1902 *Emile Duclaux*, ancien Directeur de l'*Institut Pasteur*, dans son ' Hygiene Sociale,' le plan de défense contre les infections ne doit pas résider uniquement dans la thérapeutique il faut ' mettre des barrières à leur extension ' Il vaut mieux ' placer des garde fous le long des ponts que de venir au secours de ceux qui sont tombés dans la rivière '

Or le diagnostic précoce de la tuberculose ne trouve géneralement aucun, point d'appui dans les manifestations cliniques , celles ci ne sont décelables avec netteté que plus ou moins longtemps apres l'éclosion du mal

La constatation du bacille de Koch dans les crachats ou autres humeurs de l'organisme apporte certes l'élément de certitude, mais les lesions demeurent d ordinaire, durant des années et des annees, à excrétion intermittente, et il est exceptionnel de surprendre par la bacterioscopie le début d'une tuberculose

Les expérimentateurs de tous les pays ont multiplié les recherches de labora toire susceptibles d'éclairer le diagnostic précoce Certaines de ces méthodes, par exemple la cuti réaction à la tuberculine, ont une valeur indéniable Mais aucune d'entre elles n'avait encore répondu au but à atteindre déterminer les sujets en puissance de l'infection tuberculeuse, et, au cours de cette infection, se rendre compte de leur resistance organique

La réaction qu'à récemment fait connaitre *Arthur Vernes*, la *sero floculation à la resorcine*, parait appelée, au double de point de vue que nous avons énoncé à rendre les plus grands services

La séro floculation à la resorcine répose sur des bases purement physiques, tout comme la sero floculation au péréthynol (extrait alcoolique de cœur de cheval) son ainée, qui permet de mesurer l'infection syphilitique

Lorsqu'on melange a du serum humain normal certains corps en suspension ou en solution, en faisant varier la proportion des deux elements il se produit a un moment donne une floculation Celle ci obéit a un *rythme regulier* toujours le même, s inscrivant sur un trace d'apres une courbe sinusoidale à une ou plusieurs périodes

En operant non plus avec du sérum normal mais avec du sérum pathologique, on obtient dans quelques maladies et avec certains récatifs, un *deplacement caracteristique de la courbe* Si on se tient strictement dans la zone où le serum infecte flocule et non le sérum normal, il est possible de tirer parti des constatations faites pour le diagnostic de l infection Un instrument d optique le photométre Vernes, Bricq et Yvon, permet d'apprecier les moindres variations de trouble produits dans les liquides, et d exprimer par des chiffres les resultats obtenus

La plus grande minutie doit présider au reglage de la reaction car, en plus de la nature et de la concentration des suspensions ou solutions employées, de nombreuses conditions entrent en jeu telles que le chauffage prealable du serum, la température a laquelle il faut soumettre le mélange sérum reactif, la duree du con tact apres laquelle se fait l observation

Pour trouver la *réactif* permettant de déceler l'infection tuberculeuse A Vernes et ses collaborateurs R Bricq, H Chauchard Mlle A Gager se sont adressés a une série de reactifs mineraux ou organiques des plus variés sulfocyanate ferrique, sulfates de nickel, de cuivre, de zinc, de magnesium, phénols divers et leurs derives naphtols, alcools, acides organiques aldehydes, etc Plusieurs de ces suspensions produisent, dans certaines conditions des ebauches de floculation propres a la tuberculose mais ces zones speciales de floculation sont mal limitees chevauchant sur celles obtenues au moyen de serum normal, elles sont donc pratiquement inutilisables Un diphenol la resorcine s'est par contre montre le reactif de choix et a éte adopte apres confrontation de milliers et milliers de vérifications

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La séro floculation a la resorcine est d une grande simplicite

Le sang est preleve par ponction veineuse *au sujet a examiner (ce sujet doit* etre de preference a jeun) Apres retraction du caillot le sérum (il suffit de 2 a 3 c c) est centrifugé parfois plusieurs fois, de maniere a etre parfaitement clair un serum opalescent est inutilisable un serum legerement laque n'est pas a rejeter

Dans un petit tube dit a hemolyse on introduit 0 c c 6 du serum non chauffé, puis 0 c c 6 d une solution bidistillée de resorcine pure a 1 25 pour 100 On melange par agitation sans renverser le tube On transvase *de suite* dans la cuve du photometre pour avoir la densite optique du melange On note cette premiere lecture

Replace dans son tube, qui est bouché au caoutchouc, le melange sérum resorcine est conserve a 18—20 degrés pondant 4 heures

A ce moment apres avoir désagrege les flocons formes par renversement, à trois reprises, du tube bouche, en veillant a ne pas faire de mousse, on pratique au

photomètre une seconde lecture De la densité optique notée cette fois là on retranche la densité optique du premier examen On obtient ainsi un *degré photo métrique*, la *cote tuberculeuse*, qui s'étage le long de l'échelle de 0 à 150 et même plus haut

Tous les sérums floculent, mais les tuberculeux plus que les normaux

A de très rares exceptions près, un indice inférieur à 15 est celui d'un sérum non tuberculeux et un indice supérieur à 30 celui d'un sérum tuberculeux De 15 à 30 s'étend une zône d'incertitude Il y a en effet, quelques sérums normaux qui floculent plus que de coutume, et quelques sérums tuberculeux peu hyperfloculant, généralement de façon momentanée La réaction à la resorcine constitue alors un signe d'alerte qui réclame des examens scrologiques ultérieurs

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La séro floculation à la résorcine dans la tuberculose a déjà fait ses preuves

Les Docteurs P Uffoltz et R Jacquot, l'ont appliquée à 1240 sujets des dispensaires parisiens antituberculeux La coexistence est manifeste d'un degré photométrique elevé et de symptomes toxemiques, tels que sueurs asthénie amaigrissement, qui sont sous la dépendance directe de l'activité du poison Le tuberculeux à sclérose pulmonaire me présente d hyperfloculance qu'aux périodes de reveil de l'infection, ce qui contraste avec la fixité de la cuti réaction , les hauts et les bas observés situent la marche de l infection et sont du plus grand intérêt au point de vue pronostic Et ces distingués praticiens de *l Office d'Hygiene Sociale* concluent de leurs recherches que la réaction de Vernes est vraiment ' la traduction d'une altération du sang en rapport avec le degre et l'évolution de l'infection tuberculeuse '

Le Docteur Leulher a comparé, chez plusieurs de ses malades, les renseignements fournis par la radiologie et la sero floculation Pour lui, la sérologie permet souvent d'annoncer l'invasion de la tuberculose ou une aggravation de celle ci, alors qu'on ne constate encore aucun signe radiologique ou stéthoscopique net Il a publié, à ce point de vue, des observations absolument convaincantes

En suivant les malades au moyen de prises de sang repetées, il est loisible d'établir un vrai parallelisme entre l'évolution de la tuberculose et la séro réaction à la résorcine Nous en avons rapporté un example aux *Journees medicales marseillaises* d'Avril 1927, grâce à la complaisance du Docteur Uffoltz

Un tuberculeux, à signes stéthoscopiques et radiologiques certains porteur de bacilles dans son expectoration, s'inscrit avec un degré photométrique de 77 en Octobre 1923

Par suite des soins reçus, son état s'améliore , le degré photométrique descend à 42 en Avril 1924, puis à 34 en Novembre de la même année

Re vu en Fevrier 1925, avec des lésions pulmonaires devenues fibreuses, et ayant engraissé de 6 kilos, le sujet ne marque plus que 20 de degré photo métrique

La séro-floculation à la résorcine n'est pas applicable aux seules tuberculoses pulmonaires Elle rend les mêmes services dans les cas chirurgicaux

Chez un malade du Médecin Inspecteur Troussaint, une ostéite du pubis se déclara à la suite d'une chute de cheval Les divers examens pratiqués, cliniques, histologiques, bactériologiques éliminaient le diagnostic de tuberculose La lesion ayant donné lieu à une fistule sans aucune tendance à la guérison, le sang fut examiné a l'*Institut Prophylactique* La réaction au péréthynol donna une densité optique de zéro (donc pas de syphilis) et la réaction à la résorcine un indice tuberculeux de 72 Le supplement d'enquête, qu'entraina cette réponse, consista en l'inoculation de 2 cobayes les 2 animaux contracterent la tuberculose

Une autre observation tout aussi démonstrative a été publiée par J Peyrot, de Toulouse Un Officier Colonial on retraite etait traité depuis 10 mois pour 'gommes syphilitiques' un Wassermann, après réactivation, ayant éte trouvé faiblement positif

Pour confirmer le diagnostic de lesion tuberculeuse qu'il porta Peyrot envoya le sang de son malade à *l'Institut Prophylactique* La réponse fut nette Réaction au pérethynol = zero (donc pas de syphilis), l'indice tuberculeux est elevé

Moins de 3 mois après, l'officier mourait de tuberculose pulmonaire et intestinale, des bacilles de Koch étaient trouves dans les crachats et dans le pus d'une des tumeurs

A côté de ces cas ou l'indice élevé à la résorcine a incité au diagnostic de tuber culose en l'absence de signes cliniques convaincants, d'autres existent ou, au con traire, une sero floculation normale à la resorcine permit de rectifier un diagnostic de tuberculose primitivement porte

Ainsi, chez un malade de A Vernes le chirurgien affirma une tuberculose renale, malgre la non mise en evidence des bacilles specifiques dans les urines et un indice photométrique normal de 15 Le rein suspect fut enleve La preuve fut alors faite qu'il s'agissait d'un papilleme, ayant donné lieu aux hémorragies constatées, et non d'une lésion tuberculeuse de l'organe

La séro floculation à la résorcine dans la tuberculose est indépendante de la séro floculation au pérethynol dans la syphilis Un degre photometrique elevé par résorcine chez un syphilitique indique qu'il y a en méme temps tuberculose Réciproquement, un degre photometrique par perethynol chez un tuberculeux signifie que celui ci est en outre syphilitique Une remarquable exception est cependant a connaitre dans les premiers jours de l'apparition du chancre induré, alors que l'epreuve par peréthynol ne donne encore rien, il y a séro floculation a la résorcine L'indice élevé obtenu est, dans ce cas, très éphemere Des examens à courts intervalles montrent qu'il descend rapidement et redevient normal

Toutes les recherches pratiquées jusqu'a ce jour tendent à considérer la séro floculation à la résorcine comme spécifique de la tuberculose Il est bien entendu qu'un seul examen ne suffit pas toujours pour juger un cas Il est nécessaire, comme pour la syphilis, de tracer une *courbe de l'infection* tuberculeuse Sous l'influence de conditions diverses, l'indice peut être ramené a un chiffre normal, mais cette

cote ne se maintiendra pas abaissée si le mal n est pas définitivement jugule D où la necessité de *controles successifs* du sang pour apprecier en toute certitude

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La séro réaction de Vernes a deja suscité un certain nombre de recherches qui toutes ont été confirmatives

A Buenos Ayres N Romano et P Croveri (Mai 1926) ont examiné le sang d'une centaine de malades atteints de tuberculose ou indemnes de cette affection Le diagnostic serologique a toujours éte conforme au diagnostic clinique

A New York Miss Adelaide Baylis a experimente la reaction (Mai 1927) dans le service du Docteur J Alexander Miller elle vante les resultats obtenus

A l Institut Pasteur de Paris —A Prunell (Novembre 1926) dans le Laboratoire du Professeur Calmette a étudié comparativement la reaction de fixation du complement par l antigene méthylique et la sero floculation a la resorcine Il conclut que cette derniere methode est plus sensible ' Les degrés photométriques les plus élevés ont éte observes dans les phases avancees de l infection tuberculeuse alors que la reaction de fixation fut parfois négative Par contre des indices photométriques bas furent notes chez des tuberculeux en période d accalmie evidente du mal (par exemple apres pneumothorax evoluent favorablement) alors que souvent a ce moment là la fixation du complément se montre fortement positive

V Grysez et ses collaborateurs de *l Institut Pasteur de Lille* (Juin 1927) firent des recherches analogues a celles de Prunell portant sur 172 sujets 117 tuberculeux pulmonaires et 55 personnes saines ou atteintes d affections diverses Ils concluent que la séro floculation à la résorcine est nettement superieure par sa sensibilite à la reaction de deviation Ils insistent sur la haute valeur pour le diagnostic de la tuberculose du procede de A Vernes et lui reconnaissent une valeur pronostic considerable '

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En conclusion la sero floculation a la resorcine mérite devenir une methode courante de laboratoire

Elle permet de *dépister la tuberculose* tout à fait au debut et de de *deceler les formes larvees* de la maladie Chez un tuberculeux a lésions confirmees elle permet de *suivre la marche de l infection* les examens en série indiquent la montée du degre photométrique quand le mal gagne du terrain et la descente au contraire quand l organisme résiste victorieusement

La séro floculation apparait en outre comme le *moyen scientifique de controle* qui manquait jusqu à present pour apprecier l efficacité des medicaments essayés

Enfin pour engager la *lutte sur le terrain social* la Tuberculo réaction de Vernes se prete beaucoup mieux que n importe quelle autre methode de laboratoire Une meme prise de sang permet en effet de decouvrir syphilis (reaction au pérethynol) et tuberculose (réaction a la résorcine) qui sont les deux fleaux les plus redoutables de l humanite Les Dispensaires antisyphilitiques et les Dispensaires antituberculeux pourvu qu ils possédent l outillage nécessaire

deviennent des associés fonctionnant en liaison étroite Un examen systématique, pratiqué au moment du recrutement des fonctionnaires civils ou militaires et lors de l'embauchage des ouvriers dans les centres industriels, fera reconnaître les infectés (tuberculeux ou syphilitiques) de manière à leur prodiguer les soins que nécessite leur état La lutte sociale sera ainsi assurée 'avec science et méthode,' comme le demandait E Duclaux.

INDEX BIBLIOGRAPHIQUE

DUCLAUX, E (1902) — *L'Hygiene Sociale* Alcan, éditeur

VERNES, A (1926) — Etudes sur la sérologie de la Tuberculose, nouvelle application de la séro floculation et de sa mesure par le photomètre V B Y Travaux et Publications de l'Institut Prophylactique—Fascicule IV, Maloine éditeur

JACQUOT, R et UFFOLTZ P — Séro réaction de la tuberculose pulmonaire, in Fascicule IV, pp 27 28

LEULLIER, P — Confrontation des résultats sérologiques et radiologiques dans l'exploration de la tuberculose, in Fascicule IV, pp 27 28

LEGER, M (1927) — L'infection tuberculose et la séro-floculation à la résorcine *Marseille Médical*, No 15, 25 Mai

PEYROT, J (1927) — Séro réaction de la tuberculose à la résorcine de Vernes, *Ibid*, No 3, p. 113

VERNES, A, BRICQ R, et GAGER, A (1925) — Syphilis et tuberculose, *C R Soc Biologie*, 5 Décembre, t 93, p 1425

VERNES, A (1927) — The serological measure of Tuberculosis *Amer Rev. of Tuberculosis*, t 15, p 505

ROMANO, N et CROVETI, P (1926) — La suero reacion de floculacion de Vernes para le diagnostice de la tuberculosis *Rev Med Latino-Americ*, Juillet, p 1769

BAYLIS, A (1927) — The Vernes test for tuberculosis *Amer Rev of Tuberculosis*, Avril p 500

PRUNELL, A (1926) — La fixation du complément et la réaction de la résorcine dans la tuberculose *C R Soc Biologie*, Nov, t 95, p 1319

GRYSEZ, V, PIERRET, LANGERON, BRETON et D'HOUR, (1927) — Séro floculation par la résorcine et réaction de fixation, *Ibid*, t 97, p 245

A SCHEME FOR COMBATING TUBERCULOSIS IN INDIA.

BY

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In each province a society should be started to fight tuberculosis similar to those in France, Germany and America consisting of members from non official medical men official medical men and a few of the public men who are really interested in the question of public health

The purpose of the society should be —

(1) Starting sanatoria for early diagnosis, treatment and isolation of cases of tuberculosis and creating facilities for research work

(2) Making arrangements for prophylaxis and prevention and finding out the financial requirements

(3) Starting propaganda work by cinematograph, lectures, lantern slides in order to educate the people as to how tuberculosis is propagated and how it can be effectively counteracted

Any effective plan of work requires a huge amount of money and that has been the most vexed question in all public health schemes Let us discuss how far the financial needs can be properly satisfied I have given my best thoughts over this scheme and I beg to lay this before the members of the Congress I shall be much obliged if they can help by suggesting further modification and improvement of the scheme

It is no doubt beyond the power of the public alone to launch such a big scheme unless district boards, municipalities, Government, the millowners and planters join together to make the scheme a success

First, I give below the financial requirements and I have taken Bengal as a concrete example.

Under the management of the society, a central tuberculosis hospital should be started in one of the divisions of the province with facilities for research work and for training medical men and every other division of the province should have one hospital to be built anywhere in the best place available in the division It may be argued that these hospitals should be built in the best climate suitable for treatment of tuberculosis But considering the economic condition of the people and the expense which has to be incurred for travelling from one corner of the country to another I suppose that it will not be possible for the majority of the patients to avail themselves of the benefit of these hospitals Another important factor which we should not forget is that cases come to the hospital for treatment in a fairly advanced condition and in such cases isolation from the family is more essential and important if we consider the point of view of prophylaxis In such cases the people should be given the easiest course where the expense and time in travelling should be the least No one here will contradict my view that hospital treatment is surely more effective than that at home or no treatment at all

The central hospital should consist of about 200 beds and each divisional one about 50 beds Each hospital should consist of three different wards viz —

(1) For closed and early cases
(2) For open and fairly advanced cases
(3) For patients who have improved satisfactorily and are on the way to recovery

The central hospital must have a well equipped laboratory for research work and there must be facilities for training physicians and nurses who will be placed in charge of the divisional hospitals There should also be electrical and X ray installations in every hospital The divisional hospital should have small laboratories necessary for diagnostic purposes and if possible research work

The staff of the central hospital should consist of one chief medical officer four medical graduate house physicians one pathologist and bacteriologist one lady superintendent 16 nurses one mechanic four compounders and an adequate number of menials and sweepers and each of the divisional hospitals should have one medical officer one house physician of the subordinate medical service grade four nurses one mechanic and menials and sweepers Four additional medical officers (two of medical graduates and two of the subordinate medical service grade) and four nurses should be leave extras In the central hospital a cashier and a clerk will be necessary for the office work The following may be a rough estimate of the cost In Bengal there are five divisions so there should be four divisional hospitals —

CENTRAL HOSPITAL

For food and clothes (200 beds)	Rs	4 000	0	0
Chief medical officer		1 500	0	0
Pathologist and bacteriologist		500	0	0
Four house physicians @ Rs 250		1 000	0	0
One lady superintendent (all found)		200	0	0
16 nurses @ Rs 75 each (all found)		1 200	0	0
Mechanic		150	0	0
Laboratory expenses		500	0	0
Four compounders @ Rs 50 each		200	0	0
Menials and sweepers		750	0	0
Medicines etc		500	0	0
Electricity		100	0	0
Two medical officers as leave extras		500	0	0
Two subordinate medical service men as leave extras		200	0	0
Four additional nurses on training		200	0	0
Clerk cashier and peon for office		325	0	0
TOTAL		11 825	0	0
YEARLY		141 900	0	0
Total of 1st year's expenses		247 500	0	0

EACH DIVISIONAL HOSPITAL

For food and clothes	Rs	900	0	0
One medical officer		250	0	0
One subordinate medical man		100	0	0
Four nurses		400	0	0
Mechanic		100	0	0
Laboratory expenses		100	0	0
Menial and sweepers		200	0	0
Electricity		100	0	0
Contingency		50	0	0
TOTAL		2 200	0	0
YEARLY		26 400	0	0
For four divisional hospitals for one year		105 600	0	0

The starting expenditure may be roughly estimated as —

For building the central hospital with all fittings	Rs	300 000	0	0
For four divisional hospitals		400 000	0	0
Total 1st year's expenses		247 500	0	0
TOTAL		947 500	0	0

Now where can this huge amount be obtained from? I have said that Bengal may be taken as a concrete example There are about 97 municipalities and 27 district boards in Bengal Each of the municipalities can easily contribute Rs 1,000 as donation and a recurring yearly grant of Rs 500, each district board can give Rs 10 000 as donation and a yearly grant of Rs 1,500 Calcutta Corporation can alone pay Rs 200,000 as donation and Rs 25,000 as yearly contribution Considering the importance of the matter, we may expect a Provincial Government contribution of Rs 400,000 and a yearly grant of Rs 100 000 We may also expect about Rs 200,000 from the millowners, planters and the public A Central Government grant of Rs 25,000 can easily be expected

Contribution from the 96 municipalities @ Rs 1,000 each	Rs	96,000	0	0
" , , Calcutta Corporation	,	200,000	0	0
, , 27 district boards @ Rs 10,000 each	"	270,000	0	0
, , " Provincial Government	"	400,000	0	0
, , mills, factories, plantations	"	200,000	0	0
TOTAL	"	1,166 000	0	0

This amount may be the capital to start with

The following yearly grant may be expected —

From the 96 municipalities @ Rs 500 each	Rs	48 000	0	0
" " Calcutta Corporation	"	25 000	0	0
" " 27 district boards @ Rs 1,500 each	"	40,500	0	0
" " Provincial Government Grant	"	100,000	0	0
, " public donations and yearly grant from mills, factories and plantations	"	25 000	0	0
" " Central Government	"	25,000	0	0
TOTAL	"	263,500	0	0

Taking another Rs 7,500 for excess expenditure, inspection charges etc, the total yearly expenditure will not be more than Rs 255 000 leaving a clear balance of Rs 8,500 yearly Besides a reserve of (total donation Rs 1,166,000 minus the starting expenditure Rs 947,500) Rs 219,000 remains in hand From the interest of this sum and from the yearly balance propaganda works may be carried on effectively If funds be available, new sanatoria may be started when found necessary or number of beds in the divisional hospitals may be increased

An Indian tuberculosis congress may be held every year or every alternate year to discuss the problem and to interchange views

I think this scheme may be worked out in all the provinces with some modification and alteration Even if it be found that certain provinces cannot raise such a sum, work may be started in those provinces where there are financial facilities Gradually when the people will realize the importance of the matter, public contribution may be adequately forthcoming to start on such a scheme in all the provinces

A CASE OF HUMAN TUBERCULOSIS OF THE CERVICAL GLANDS CAUSED BY THE AVIAN TUBERCLE BACILLUS

BY

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In Charge Bovine Tuberculosis Enquiry Indian Research Fund Association
(From the Imperial Institute of Veterinary Research Muktesar)

THE avian tubercle bacillus is generally held to be non virulent for man and it is believed to play no important part in human tuberculosis Cases are on record however in which infection with this bacillus has in rare instances been found in man Lowenstein found avian bacilli in sputum and described two cases of tuber culosis of the kidney in children caused by avian bacilli Weber isolated avian bacilli from the fæces of a phthisical patient Max Koch and Rabinowitsch culti vated them from the spleen pulp of a man dead of miliary tuberculosis Kruse has recorded three cases Pansini one Lipschutz one and Janeso and Elfer one in which avian bacilli were found infectious for man More recently two similar cases have been recorded from the Mayo clinic There are altogether about 20 instances in which this bacillus has so far been found in cases of human tuberculosis (1)

The object of this paper is to place on record the occurrence of tuberculosis of the cervical glands in a child caused by the avian tubercle bacillus The case was met with in the course of investigation into the nature of organisms causing surgical tuberculosis in human beings in India The material for investigation was received from the Cama Hospital for Women and Children Bombay through the kindness of the physician in charge Dr (Miss) E Turner Watts who supplied the following history of the case

A girl D aged 14 years was suffering from enlarged glands in the neck for about two months The glands were found to be all matted together and were situated in the anterior and posterior triangles on the right side of the neck The patient was fairly well built and had no cough She sought admission into the hospital for the complaint and the glands were removed by operation A portion of the material was sent at my request to the laboratory for investigation

The specimen consisted of enlarged glands varying in size from a large pea to a small walnut which on section were found to be entirely caseous Examination of smears made from the glands did not reveal any tubercle bacilli The caseous portion of one of the glands was emulsified and inoculated subcutaneously into a guinea pig This animal died after six weeks but unfortunately was not available

for further investigation as the carcase was wholly devoured by rats Another guinea pig had been inoculated with the same material a week after receipt of the specimen the emulsion this time was treated with *normal* sodium hydroxide solution according to a method previously described (Soparkar, 1916) This guinea pig was sacrificed but no naked eye lesions of tuberculosis were found in the animal except a small pea sized abscess at the seat of inoculation which showed a few tubercle bacilli on microscopical examination Further passage was done through another guinea pig which was inoculated with the material from the previous animal This guinea pig died after 65 days Post-mortem examination did not reveal any evidence of tuberculosis in this animal also except a small collection of thickish pus at the seat of inoculation which showed no tubercle bacilli Yet another passage was made and a fresh guinea pig was inoculated with an emulsion of pus and spleen from the previous animal This guinea pig died after 75 days In this animal one of the lumbar glands contained a minute caseous focus which on microscopic examination showed a few tubercle bacilli but no other evidence of tuberculosis was detectable

Attempts were made to isolate tubercle bacilli from this small lesion in the lumbar gland and several tubes of egg medium without glycerine were sown with the material In about four weeks' time minute colonies made their appearance in some of the tubes which at first gave an impression of contamination as they appeared moist and translucent and lacked the usual dull dry character noted in cultures of mammalian tubercle bacilli Subcultures on serum agar gave in two weeks a thin moist translucent film which also appeared as if it were a contamination Microscopical examination of the smears showed numerous acid fast bacilli mixed with a number of non acid fast rods Whether these non acid fast rods were tubercle bacilli or other contaminating organisms it was difficult to say, although in morphological appearance they resembled the other acid fast bacilli The purity of the culture was then tested on ordinary agar This failed to show any growth of contaminating organisms indicating that the culture was pure and that the non acid fast bacilli were also tubercle bacilli probably young The purity of the culture and the character of the growth raised a suspicion that the strain might possibly be of the avian type although this was not suspected before owing to the rarity of such an occurrence It was therefore sown on glycerinated media—glycerine serum agar and glycerine egg On these media the culture produced a thick moist almost slimy growth which when scraped from the medium and rubbed up in a mortar with physiological salt solution produced an homogeneous suspension with characteristic ease

Animal tests were further done and the culture was tested on rabbits and fowls Two rabbits were inoculated with 1/10 mg and two with 1/100 mg of the culture intravenously Although the rabbit is susceptible to both the bovine and the avian types of tubercle bacilli, intravenous injection of the latter type usually produces in this animal early death with multiplication of bacilli and without the production of anatomical lesions—a septicæmic form known by the name of the type of Yersin

The post-mortem appearances are different from the characteristic lesions produced by injection of the bovine bacillus and it is thus possible to differentiate between the two types Again the animal being insusceptible to the human type serves also to distinguish between the human and the avian types All the rabbits died, as a result of inoculation, those injected with 1 100 mg in 29 to 40 days, and those with 1 10 mg in 20 to 23 days respectively In no case were lesions observed resembling those usually produced by infection with the bovine bacillus (Table I)

TABLE I

Experiments on rabbits with a strain of human origin (Bombay C H I)
(Intravenous inoculations)

Number of Rabbit	Date of inoculation	Age and generation of culture	Dose in milligrams	Weight of Rabbit in grammes		Duration of life in days	Post mortem results
				Initial	Final		
209	9-7-27	21 days old first generation	0 01	1 850	1 300	D 29	Lungs bright pink soft crepitant No tubercles detected Liver enlarged and congested Spleen swollen and soft All lymph glands swollen No naked eye evidence of tuberculosis detected Smears showed many tubercle bacilli in the spleen and some in most of the organs and glands
210	9-7 27	Do	0 1	1 780	1 200	D 23	Lungs pink soft crepitant show numerous small areas of congestion No tubercles detected Liver enlarged and congested and shows a few coccidia nodules Spleen swollen and soft Kidneys show areas of congestion Many lymph glands swollen Many tubercle *bacilli in bone marrow* and the spleen and some in most of the other organs and glands
211	9-7 27	Do	0 01	1 750	850	D 40	Similar to Rabbit 210
212	9-7 27	Do	0 1	1 720	1 300	D 20	Similar to Rabbit 210

K = Killed
D = Died

TABLE I—*concld*

Number of Rabbit	Date of inoculation	Age and generation of culture	Dose in milligrams	Weight of Rabbit in grammes — Initial	Final	Duration of life in days	Post mortem result
290	2-8-27	25 days old second generation	0 01	1,500	1,190	K 95	Lungs, soft, crepitant Scattered pin head sized tubercles seen. Liver shows some coccidia nodules Spleen moderately enlarged, no tubercles seen. Kidneys show many minute grey foci on the surface Glands slightly enlarged Joints normal Microscopic examination—no tubercle bacilli detected
291	2-8-27	Do	0·01	1,150	930	D 38	Very numerous tubercle bacilli in the spleen, otherwise *similar to Rabbit 209*
292	2-8-27	Do	0 1	1,350	860	D 28	Very numerous bacilli in the spleen and the liver, otherwise similar to Rabbit 210
293	2-8-27	Do	0 1	1,400	630	D 55	Lungs moderately enlarged and pale, showing broncho-pneumonic condition No tubercles detected. Liver enlarged and shows many coccidia nodules Spleen swollen and soft and shows a few greyish foci All glands *enlarged Smears showed* very numerous tubercle bacilli in the lungs, liver, spleen and the bonemarrow and many in the lymph glands
334	17-8-27	15 days old, third generation	0 01	1,310	810	D 26	Lymph glands normal. Very numerous tubercle bacilli in spleen, otherwise similar to Rabbit 209
335	17-8-27	Do	0·01	850	1,140	K 81	No evidence of tubercle detected Microscopic examination—no tubercle bacilli seen
336	17-8-27	Do	0 1	1,360	850	D 15	Lymph glands normal, otherwise similar to Rabbit 209
337	17 8-27	Do	0·1	1,390	1,050	D 40	Rabbit died of hæmorrhage in the pleural cavity, otherwise similar to Rabbit 293

K.=Killed

D.=Died

One fowl was inoculated intravenously with 1/10 mg of the culture This died in 24 days On post mortem examination, the liver and the spleen were found considerably enlarged and a few necrotic patches varying in size from 2 to 5 millimetres were seen on the surface of the liver Smear examination from these organs showed very numerous tubercle bacilli, many of them being present in characteristic rosettes

The animal experiments thus confirmed the suspicion about the avian character of the isolated strain In order further to confirm this finding, additional batches of rabbits and fowls were inoculated Of the eight rabbits inoculated all except two died in 20 to 60 days with characteristic appearances of acute avian infection Three more fowls that were inoculated with this strain, two with 1/10 mg and one with 1 mg intravenously, died, all within 22 to 30 days of severe septicæmia and great multiplication of bacilli, smears from the organs showing bacilli in most cases in very large numbers and many of them in characteristic clumps

TABLE II

Experiments on fowls with a strain of human origin (Bombay C H V)
(Intravenous inoculations)

Date of inoculation	Number of Fowl	Age and generation of culture	Dose in milligrams	Duration of life in days	Post mortem result
9 7-27	17	21 days old first generation	0 1	D 24	Liver much enlarged Some necrotic patches 2 to 5 mm sq on the surface Spleen enlarged and dark red in colour Smears show numerous tubercle bacilli majority in large clumps A few bacilli found in the bone marrow none in the lungs and kidneys
2-8 27	19	25 days old second generation	0 1	D 22	Liver slightly enlarged and shows scattered grey foci on the surface Spleen normal Smears from both show numerous tubercle bacilli
2-8-27	20	Do	1	D 30	Liver moderately enlarged and shows a few grey foci on section Spleen enlarged dark red in colour and shows several grey foci on the surface Smears show very numerous bacilli in each, mostly in clumps Kidneys bone marrow and lungs show some bacilli
17-8-27	22	15 days old third generation	0·1	D 27	Liver and spleen normal in appearance Smears show many bacilli in the liver and numerous in the spleen with several large clumps A few bacilli in the kidneys and the bone marrow

D = Died

Study of the cultural characters and results of infection of rabbits and fowls left no doubt as to the nature of the strain which was avian

Further confirmatory tests were made by isolating strains from the inoculated animals after their death and study of their characters A strain was isolated from one of the rabbits that died 40 days after inoculation and another from the fowl Both these on cultural tests gave the growth characters of the avian type Two guinea pigs were inoculated subcutaneously with a large dose (10 mg) of the culture One of the animals died after seven weeks and the other after nine weeks. Post mortem examination showed only a small local abscess at the seat of inoculation and enlargement of the spleen but not the generalized progressive tuberculosis characteristic of inoculation with mammalian tubercle bacilli The result further confirmed the character of the strain

A point of interest arises from these results—

The persistent failure of the series of guinea pigs inoculated with the material from the caseous glands to develop definite tuberculosis is significant in view of the later findings in regard to the nature of the infecting organism which was avian and the fact that this animal is very resistant to infection by the avian bacillus This failure in the ordinary course would have been attributed to the tubercle bacilli in the original material being dead or devitalized and the case would have been grouped as such

Several such instances are on record in which inoculation of the guinea pig with tuberculous material gave negative results

Griffith(5) had met with seven instances in which injection with caseous material from glands failed to produce tuberculosis in guinea pigs

The same investigator(4) working at Cambridge met with five instances out of 40 in which no living bacilli could be recovered from lesions which to the naked eye appeared tuberculous

Weber(8) reported 17 cases in which caseo calcareous glands found in children were injected into guinea pigs without causing tuberculosis

Eastwood and F Griffith(3) have recorded 16 cases out of 94 examined in which tuberculous lesions in children on injection failed to cause infection in the guinea pigs

Among 17 cases of cervical gland tuberculosis examined by Lewis(6) in two instances injection of the glandular material failed to infect the guinea pigs

Cobbet(2) when working for the Royal Commission found that definitely caseous nodules from the lymphatic glands of children might be incapable of infecting such a susceptible animal as the guinea pig although the material might contain plenty of well formed tubercle bacilli

The number of instances in which evidently diseased tissue containing tubercle bacilli failed to provoke tuberculosis in the guinea pig is important in view of the great susceptibility of this animal to mammalian tubercle bacilli and the fact that an extremely small number of these organisms is sufficient to set up the disease in the animal In contrast to these findings there are numerous instances on record in which tuberculosis was produced in guinea pigs by injection of material from

apparently normal glands and in some of which even histological changes were not detected

Could the failure in the instances mentioned previously have been due to the possibility in some cases at least of the infecting organism being of the avian type?

That the guinea pig which is the most common laboratory animal used for the isolation of tubercle bacilli is, as has already been noted very resistant to infection by the avian bacillus and inoculation of the guinea pig in cases of this infection often gives negative results while a positive result is obtained if one employs the fowl

Although cattle are supposed to be not very susceptible to infection by the avian bacillus a number of instances have recently been recorded in Denmark in which abortion in cows was found to be caused by the avian bacillus Again in America(9) investigation into the cause of increase of tuberculosis among swine has shown that in a very large proportion of cases the infection is caused by the avian bacillus

The case described in this paper and those cited in the early part shows that tuberculous infection in the human subject may be caused by the avian bacillus

Due regard to this possibility and a systematic employment of the fowl along with the common guinea pig in investigation of this nature will throw more light upon the incidence of infection by the avian bacillus in human beings

ACKNOWLEDGMENTS

I wish to express my great indebtedness to Dr J T Edwards Director Imperial Institute of Veterinary Research Muktesar for much valuable help and numerous facilities afforded for carrying out the work at the Institute

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REFERENCES

(1) CAMERON T W M (1916) Diseases of Animals in Relation to Man Faber and Gwyer Ltd London 1916 p 46

(2) COBBETT L (1907) Roy Com Tuberculosis 2nd Int Report App Vol II p 17

(3) EASTWOOD and GRIFFITH F (1914) Supplement to the 42nd Annual Report of the Local Government Board 1912 13 p LXVI

(4) GRIFFITH A S (1914) *Ibid* p LXVII

(5) *Idem* (1911) Roy Com Tuberculosis Final Report App Vol I p 18

(6) LEWIS PAUL A (1910) Tuberculous Cervical Adenitis *Jour Exp Med* Vol XII p 85

(7) SOPARKAR M B (1916) The Cultivation of the Tubercle Bacillus directly from Sputum and Post mortem Material *Ind Jour Med Res* Vol IV p 28

(8) WEBER A (1906) Die Infection des Menschen mit den Tuberkle bacillen des Rindes (Perlsucht Bacillen) *Deut Med Woch*, No 49 p 1982

(9) VAN ES L and MARTIN H M (1925) An Inquiry into the Cause of the Increase of Tuberculosis of Swine *University of Nebraska Agricultural Experiment Station Research Bulletin* 30

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(1) CAMERON T W M (1916) Diseases of Animals in Relation to Man Faber and Gwyer Ltd London 1916 p 46

(2) COBBET L (1907) Roy Com Tuberculosis 2nd Int Report App Vol II p 1

(3) EASTWOOD and GRIFFITH F (1914) Supplement to the 42nd Annual Report of the Local Government Board 1912 13 p LXVI

(4) GRIFFITH A S (1914) *Ibid* p LXVII

(5) *Idem* (1911) Roy Com Tuberculosis Final Report App Vol I p 18

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(7) SOPARKAR M B (1916) The Cultivation of the Tubercle Bacillus directly from Sputum and Post mortem Material *Ind Jour Med Res* Vol IV p 28

(8) WEBER A (1904) Die Infection des Menschen mit den Tuberkelbacillen des Rindes (Perlsuchtbacillen) *Deut Med Woch* No 49 p 198

(9) VAN ES L and MARTIN H M (1925) An Inquiry into the Cause of the Increase of Tuberculosis of Swine *University of Nebraska Agricultural Experiment Station Research Bulletin* 30

DISCUSSION

Major A Parker Hitchens (Philippine Islands) (Chairman) said The conditions in the Philippines were different, the incidence was much more frequent than in India

He was greatly interested in the papers by Dr Ukil and Dr Ghosh and thought that the Bureau of Education ought to do the most important work of propagating knowledge He thought promiscuous spitting was one of the most important causes of the spread of tuberculosis in the tropics Nutrition in the earlier ages was most important in building up the resistance of their bodies to invasion by the tubercle bacillus The mothers should be educated to educate the children, as they are most amenable to such education The Bureau of Education ought to introduce teaching on this subject and other matters of public health into schools and to teach these subjects in the same way as they do one of the popular sports He laid great stress on the teaching of public health in schools

Dr C Frimodt Moller (Madras) The campaign against tuberculosis should not wait until the condition of general hygiene has improved For in the campaign in Europe the death rate in France did not decline at the same time as the hygienic conditions improved, while the death rate fell fast in England and other countries where a direct attack on the disease was begun France only took up such an attack on similar lines to the other countries after 1914 The campaign should be isolation and education, isolation in sanatoria and hospitals It has been pointed out before, last of all in the *Indian Medical Gazette* (June 1926) that not only should a central sanatorium with research facilities be established in each province, but, when the doctors and students have been trained, a chain of smaller cheap hospitals near each city and town Only after this should the dispensary come in as a clearing house for the hospitals and sanatoria

Dr Robert J Gittins (Central Provinces) The present measures for our fight against tuberculosis in India are quite inadequate when we consider the magnitude of the problem I hope that this meeting will be the beginning of a wider, more intensive and co ordinated campaign against the disease I strongly support Dr Frimodt Moller s view that in addition to well equipped first class sanatoria one great need is for cheap tuberculosis hospitals close to the larger towns providing accommodation suitable for the type of patients Here patients will be diagnosed, appropriate in patient treatment instituted, the 'tuberculosis' life inculcated and certain patients will be sent on to the sanatoria They will be centres of propaganda and education for the masses We must guard against the propaganda lecture being too elaborate, which mistake is some times made It is needless to emphasize the need for children to be taught the essentials of hygiene, on which they are at present very ignorant My experience, contrary to that of our chairman, is that (at least in my part of the country,—the C P) the Indian tuberculous patient, when put to rest and treated on general lines on the plains does not
pro
out
at
cases of phthisis, which at present only too often go downhill in spite of the best available general treatment We must see that practitioners generally are thoroughly

instructed in the use of this form of treatment, including its indications and contra-indications This treatment has a close relation to prevention, in that by a more extended use of it, we shall begin to show far better results, and patients will be attracted to the tuberculosis hospitals of the plains, which, as I have said, are so badly needed At risk of repetition I should like to see some form of definite pronouncement from this Congress as to the need of a scheme for much more intensive and co ordinated research into tuberculosis in India as we have in the case of other diseases

Dr S Sarbadhikary (Bengal) Tuberculosis is spreading in Bengal so rapidly that even if Dr Ghosh's dream turned out to be a fact to morrow, it would be inadequate to tackle the problem I agree with the chairman that education among children about public hygiene is more important This education should be carried on not only by a group of teachers, but by general medical practitioners as well The general practitioner should not think that his duty finishes with the treatment of the patient who comes under his treatment, but it is his first and foremost duty to educate the people of the family in the methods of preventing the spread of infection to other members of the family As in Bengal it will not be possible for various obvious reasons viz financial, etc, to have, in the near future, an adequate number of sanatoria and hospitals for the proper isolation of tubercle bacillus cases, we shall have, in the meantime to pay more attention to individual isolation, disinfection of excreta, arrangement of separate utensils, etc Considering the fact that the socio-economic factor cannot be solved in a short time, we should pay more attention to the active treatment of the victims and their isolation As regards the proper treatment of the disease early diagnosis is one of the most important points, and as the mofussil practitioners lack up to date knowledge in diagnosis and treatment, it would be better to organize an institution where the practitioners may unite, have interchange of thought and learn the progress of modern scientific methods from time to time The proposal for this institution is no reflection on the ignorance of the general practitioners, but is made on the same principle as that of this Congress, only on a smaller scale The artificial pneumothorax treatment should be more popular, and there should be an arrangement to train people in this line, as this method has proved more satisfactory than any other and has not been so much in vogue in this country

Major J J Harper Nelson, I M S (Punjab) I am taking part in the discussion [illegible]

tuberculosis As a teacher in a large medical school it has been my privilege constantly to impress on students the importance of early diagnosis and treatment In addition, I have recently had the privilege of presiding at a meeting of the Society for the Propagation of Scientific Knowledge in Lahore, a society originally started by medical students and now devoted to propaganda of scientific facts amongst the general public The subject was the prevention of the spread of tuberculosis and was well attended, the audience consisting of 75 per cent of school boys I think such propaganda is to be encouraged We cannot make bricks without straw and our difficulty is that the organizing of efficient propaganda for the education of the people and also the organization of means of treatment is hampered by gross lack of funds As regards treatment

I favour the establishment of small efficient tuberculosis hospitals as a first step and later developing sanatoria to which suitable cases can be drafted to complete treatment begun in the local hospitals *I also favour developing the tuberculosis dispensary*, on the lines of the Edinburgh scheme, where cases can be discovered in the early stage and drafted into hospitals for treatment From this dispensary preventive propaganda could also be sent out *The question of treatment by artificial* pneumothorax is, I think, outside the province of this discussion, but would merely state that over five years' experience of its use has convinced me of its utility

In conclusion I would suggest that at the next Congress the question of tuberculosis be given a more prominent place in our deliberations We have days given to the discussion of malaria, kala azar, leprosy, filariasis, etc , whereas tuberculosis has been *relegated to a single session*

Dr R K Kacker (United Provinces) The tuberculosis problem has two aspects, viz , treatment and prevention Sanatoria and tuberculosis hospitals may help to solve

raised by removing or reducing poverty providing better houses and more adequate, wholesome and nutritious food, and doing away with certain pernicious social customs the problem of tuberculosis control and eradication will remain unsolved A sanatorium is certainly a valuable measure in the campaign against tuberculosis but I do not lay so much stress on it as my esteemed friend, Dr Frimodt Moller Sanatoria are more expensive to build and expensive to maintain, especially in the hills I am, therefore, more in favour of starting tuberculosis dispensaries in all the large municipalities to begin with They would act as propaganda treatment survey centres as suggested by Dr Muir in connection with the campaign against leprosy They could carry on treatment on the class method so strongly advocated and *successfully* carried out by Dr Ball of America Money is scarce in India and, to begin with, we should only adopt the least expensive and most practicable measures which are likely to yield the maximum of useful *results* Treatment by artificial pneumothorax is undoubtedly very useful in a certain type of case, but its applicability is limited and its application has certain drawbacks It should not, therefore, be undertaken light-heartedly outside an *institution, where trained workers in this mode of treatment* are not available As a suggestion has been made for co ordination of anti tuberculosis efforts I beg to make a specific proposal, viz , that a society of medical men engaged in *anti tuberculosis work and connected with tuberculosis institutions*, be formed on the same lines as the society of superintendents of tuberculosis institutions in England

Dr J Banerji (Bengal) *Refuted the ignorance of the general practitioner* but also thought the chief difficulty was as regards socio economic factors, the joint family system and above all the ' purdah ' system

Dr E R Webb (Bihar & Orissa) Dr Kacker *has reminded us of the incessant* demand on our humanity for the care of patients already sick In laying stress on the need for local tuberculosis hospitals he voices my own feeling that tuberculosis must be dealt with locally But the problem *of building hospitals and of staffing them* remains

In the sanatorium we treat patients, we teach them and we teach friends, nurses and doctors This treatment is a definite accomplishment The education is extensive i e, those educated must pass on their knowledge as they are able, and show the burden of responsibility for prevention of tuberculosis

In a sanatorium, more than in any other place the problem of tuberculosis is vividly realized by all Experience in general practice enhances this appreciation We need sanatoria

I heartily endorse all that Dr Frimodt Moller has said Further, may I point out the need of educating the student, as well as the practitioner? Realizing the educational value of an efficient sanatorium the University of Minnesota sends *all* its medical students to Glen Lake Sanatorium, a 600 bed sanatorium dealing with all types of tuberculosis for a three weeks' clerkship some of these use the opportunity to return there during their intern year Such facilities are not always available Dr Krause, addressing the National Tuberculosis Association in 1926 pointed out the demands that are made on the student's time by the many departments which feel that special instruction must be given eyes nervous diseases dietetic diseases, etc, etc From his considerable experience in teaching he said that at the least, competent instruction should be given to all medical students in tuberculosis in the out patient department of his or her medical college hospital

Capt P Ganguli, I M S (Bengal) In connection with the problem of prevention of tuberculosis in India which has assumed such alarming proportions various schemes have been put up by several speakers I do not know how far vaccination as recommended by Calmette, will be useful in this country but it is supposed to give an acquired immunity In civilized countries there is no doubt that the natural immunity of the population is raised by means of mild infections and subsequent cures In India, however massive infection is responsible for the spread of the disease

The destruction of the lipoid or waxy armour of the tubercle bacillus is a point of considerable importance I consider that this factor plays a very important part in any question of natural immunity In a series of 156 cases of pulmonary tuberculosis the amount of serum lipase, which according to Rowntree's method works up to from two to three in normal healthy people, was invariably below two in tuberculous patients This diminution in the serum lipase has an important bearing on the prognosis of the patients, for those patients who improve under any method of treatment be it by the fatty acids of Sir L Rogers or by the more recent gold treatment with krysolgan or sanocrysin, always show a subsequent increase in the lipase content of the serum In my experience with sanocrysin, I have been struck with the inconstancy of its action. In certain selected cases, the improvement has been remarkable and in others, there has been no apparent benefit at all In these latter cases the serum lipase has been invariably below two in spite of treatment In Bengal the serum lipase is deficient in the majority of cases and this I attribute to the want of vitamin A containing substances in the dietary The people are so poor that the majority live on half their normal subsistence diet, and even this diet is one sided and wanting in protein and fats The price of milk and fish is increasing daily in Bengal for their supply is diminishing while the population is increasing This is the economic problem which calls for

attention if we want to raise the natural immunity of our people and diminish the prevalence of tuberculosis in India

Dr M B Soparkar (India) Dr Ukil referred to cases of surgical tuberculosis, viz, glandular tuberculosis, bone and joint tuberculosis, abdominal tuberculosis as occurring in Bengal This form of tuberculosis is found to be fairly common in India As to its causation, in European countries, where tuberculosis among cattle is common, surgical tuberculosis in human beings is found to be caused, in a large proportion of cases, especially in young children, by the bovine bacillus through infected milk, as is shown by the work of Mitchel, Fraser, Griffith, Park and Krumweide, and others In India, tuberculosis among cattle is generally held to be rare (about three per cent) but very little work on the nature of surgical tuberculosis has so far been done in this country In a paper read before the Indian Science Congress in 1925 I gave the results of an investigation of 65 cases, comprising 40 cases of cervical gland tuberculosis, eight cases of axillary gland tuberculosis and 17 cases of pulmonary tuberculosis, and in no instance was the disease found to have been caused by the bovine bacillus Recently, on examination of carcases at the slaughter-house at Ferozepur and Lahore in the Punjab, I have found that the disease is more frequent and occurs to the extent of over 14 per cent, an incidence approaching that found in some places in Europe The findings would call for a systematic survey of the animal disease in different provinces and an investigation into the nature of organisms causing surgical tuberculosis in these parts

BACTERIOLOGY.

A COMPARATIVE STUDY ON LEPTOSPIRÆ.

BY

PROF R INADA,

Tokyo Imperial University.

FRIDAY DEC 9TH, 10 A.M. TO 1 P.M.

THE author will present the results of the investigation made in his laboratory on the biological differentiation of *Leptospira icterohæmorrhagiæ hebdomadis* A and B types, *icteroides*, *febrilis*, and water leptospira

On the resistance of the leptospiræ against various external influences, Dr S Anjo studied the oligo dynamic action of metals and the symbiosis with other bacteria, the resistance against saponine bile bile acid salt, organic and inorganic acid with the following results —The pathogenic and water leptospiræ are different in their resistance in relation to the oligo dynamic action and the symbiosis with other bacteria The resistance of pathogenic leptospiræ is weaker than that of water leptospiræ Thus the pathogenic leptospiræ are divided into two groups The one, to which the *L icterohæmorrhagiæ* and *icteroides* belong is weakest in its resistance The other, to which *L hebdomadis* A and B types and *febrilis* belong is stronger in resistance than the former, although it is weaker than the water leptospira

For the immunological study Dr S Shimozawa took up various sources of leptospiræ as follows —Agglutination tests culture in the immune serum, Pfeiffer's phenomena, the protection tests with immune serum and the protection tests with active immunization The immunological differences of *L icterohæmorrhagiæ* and *hebdomadis* will not be mentioned here as they were already reported in the last Congress The author could find no noticeable difference between *L icterohæmorrhagiæ* and *icteroides* The foregoing results of the oligo-dynamic action on them seem to coincide with this result *L febrilis* is able to agglutinate with the immune sera of *L icterohæmorrhagiæ* and *icteroides*, even if in lesser degree of dilution and the immune serum of *L febrilis* can agglutinate *L icterohæmorrhagiæ* and *icteroides* In the protection tests with the active immunization, the author could not differentiate L *febrilis* from *L icterohæmorrhagiæ* and *icteroides* From the standpoint of the oligo dynamic phenomenon *L febrilis* seems to belong to the group of *L hebdomadis*, while it belongs to the *L icterohæmorrhagiæ* from the immunological findings

COLOUR VARIATIONS IN THE FUNGUS OF DHOBIE'S ITCH (*EPIDERMOPHYTON CRURIS*)

BY

C McGUIRL D T M

School of Tropical Medicine and Hygiene Calcutta

Dhobie's itch is a special type of ring worm commonly met with in the tropics and caused by the fungus *Epidermophyton cruris* It may attack any part of the glabrous skin but has never been known to affect the hairy areas Previously the fungus has never been cultivated in India and our knowledge has been confined to textbook descriptions

During the last eighteen months whilst working under Lieut Col H W Acton in the skin clinic School of Tropical Medicine and Hygiene Calcutta I have successfully cultivated nine variations of this fungus

The object of this paper is to show that the *Epidermophyton cruris* is a single species with many colour variations

Method of cultivation—To obtain a successful culture of *Epidermophyton cruris* it is essential to collect those scales which contain mycelia and these are best obtained from the advancing edges of the eruption In conditions like cheiropompholyx where vesicles are present the top of the vesicle is cut off and then used for cultivation It is best to select those vesicles which only contain serum and are not purulent The difficulty in obtaining primary cultures is due to the number of other organisms such as yeasts staphylococci and spore forming bacilli which are commonly found on the skin These secondary organisms grow more rapidly than the epidermophyton and hinder its growth To prevent any secondary organisms from growing the effect of drying the scales was first tried The scales were placed between two sterile slides and left in a desiccator for eight days and then used for cultivation Though this method did hinder the growth of secondary organisms yet in some cases where aspergilli were present all the scales were contaminated by the growth of these fungi

The scales were then exposed to direct sunlight for two hours before cultivation but this did not prove successful Gentian violet 0 004 per cent was then added to the media to inhibit the growth of these secondary organisms This method did hinder the growth of organisms but the epidermophyton grew pleomorphic in character and we were unable to study it So far the best results have been obtained by soaking the scales in absolute alcohol for ten minutes After this they are directly

planted on Sabouraud's maltose agar About seven tubes are used and five plants are made on each tube On an average six positive growths are obtained from 35 plants In some cases a growth of the epidermophyton was obtained after 25 minutes soaking in absolute alcohol By the fourth or fifth day the growth of the Epidermophyton is visible as a small downy area 2 to 3 mm in size, and sending down small Medusa like roots into the media Any fungus which grows before the fourth day, one may safely say is not the epidermophyton

Variations in the growth of the epidermophyton —Up to date nine variations of the epidermophyton have been cultivated on Sabouraud's maltose agar These are best seen in primary or early subcultures as with age some of these variations lose their pigment In making subcultures the material should be selected from the growing edge of the fungus where surface runners only are present If the material is taken from the downy central area, the subculture is always pleomorphic The variations in the growth consist of differences in (1) the colour (2) the presence or absence of downiness, (3) the number of concentric rings, and (4) the character of the radial furrows

(1) The variations in colour varied from growths which had no colour to growths which were yellow or orange to reddish purple In Sabouraud's maltose agar the colour was not always the same Sometimes the primary growths were coloured and the secondary growths were devoid of colour, whilst at other times the reverse held good The variations were, therefore, studied on the following media On glucose agar a purple pigment was produced by all the variations, and in some cases the pigment extended into the media On ordinary agar all the growths had a slight lemon tint On Dorset's egg with glycerine the growths were a deep purplish colour On 2 per cent saccharose some growths showed an orange colour whilst others were lemon yellow On carrot some were faintly brick red, whilst others had no colour at all

It is by studying these colour variations on the above media that one is able to distinguish a yellow culture from an orange or red one Further it will be seen that these differences in colour are not due to variations in the species of the fungus, but are dependent on chemical substance present in the media

(2) Downiness may be present in some growths over the whole surface, whilst in others it may be limited only to the central area or may be totally absent The presence of down is also largely influenced by the chemical substances present in the media, and it is most marked on glucose agar and least on Dorset's egg Moisture is another factor which determines the growth of down and the drier the media the more down is produced It is best when studying these fungi to inoculate the tubes three days after they have been prepared

(3) The concentric rings are present in all variations, and are more numerous in some growths than in others They are caused by the centrifugal spread of the growth from the centre, and are best seen on Sabouraud's maltose agar These rings correspond to the yearly rings of growth seen on section of the stem of a tree.

(4) The radial furrows vary in number in the different variations and may be either confined to the centre or extend for some distance to the periphery They are best seen in old cultures and are due to the roots contracting and infolding the surface of the growth These furrows are best brought out on glucose agar

These nine variations were then planted on a synthetic medium devised by Lieut Col H W Acton, I M S, which consisted of saccharose and the amino-acids tryptophane and arginine nitrate In this medium all these variations grew in subculture without any variation in colour and appearance, thus showing that they all belonged to a single species

The morphology of the fungus —The morphology of the epidermophyton was studied by (1) examining the aerial hyphæ and end organs (2) examining the surface runners and (3) cutting sections of agar cultures to study the roots

(1) The aerial hyphæ and end organs were studied by making hanging drop preparations of the fungi, which were prepared in the following manner A deep well slide and coverslip was taken and sterilized in the autoclave A large drop of Sabouraud s maltose agar which had previously been melted at 100°C was then taken on a platinum loop and placed on the coverslip The edge of the coverslip was then smeared with vaseline and the coverslip placed over the slide with the agar surface downwards After 24 hours when the agar had solidified it was inoculated with the fungus to be examined and the slide was kept in the dark After one month it was examined with the 1/6th objective, and the following end organs which are present in all variations of the epidermophyton were seen

The first is a segmented spindle which is situated at the end of the aerial hyphæ and is called ' fuseaux ' by French writers The second are the spores or conidia which are round or oval in shape and are situated along the hyphæ These spores or conidia may be either arranged in clusters like a bunch of grapes or singly along the hyphæ When these spores are arranged in clusters they are called grapes, and when arranged singly along the hyphæ are known as hyphes sporiferes simples The third type of end organ is a curling of the end of the hyphæ called a tendril These look just like the tendrils of creepers Sometimes the hyphæ at the end of this tendril start growing and produce knots along the mycelium All these end organs may not always be present in the same hanging drop preparation, in some variations as many as eight slides were examined before all varieties could be found

(2) The surface runners were examined by scraping off the aerial hyphæ These grow from the centre in a centrifugal manner and consist of segmented and non segmented mycelia

(3) The deep roots were studied by examining the cultures from the side and making sections of young agar cultures When viewed from the side, the roots of all the variations of the epidermophyton appeared fine and diaphanous like a jelly fish which extended deep down into the media Fresh hand sections were made by breaking the test tubes and then cutting transversely through the media with a Gillette blade These sections were then stained by weak carbol fuschin and

mounted with euparal after 24 hours When examined with the 2/3rd objective, the deep roots were seen penetrating in a radiating manner deep down into the media When examined with the 1/6th objective, the roots were seen to consist of young non segmented and coarser segmented mycelia

It will be seen by this morphological study that these variations of *Epidermophyton cruris* all have the same type of roots surface runners and end organs, and should be classified as a single species The differences in colour, the presence or absence of down, the character of the radial furrows and the differences in the number of concentric rings are factors which are influenced by physical conditions, as well as by variations in the chemical substances present in the media and are, therefore, variant characters of the fungus The morphological characters should, therefore, first be studied before differentiation into different species is made

Conclusions

(1) The *Epidermophyton cruris* is a single species with many colour variations

(2) Nine morphological and colour variations of this epidermophyton have been cultivated by us

Acknowledgments

My thanks are due to Lieut Col H W Acton, I M S, for his valuable help and advice through which it was possible to do this work

THE MALASSEZIA OF THE SKIN, THEIR CULTIVATION, MORPHOLOGY AND SPECIES

BY

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WHEN from a case of dandruff (*pityriasis simplex capitis*) a scale is examined under a microscope, large numbers of yeast like bodies are found The microscopic field presents such a remarkable picture that one is tempted to ask what the organism will look like in culture on artificial laboratory media The organism of dandruff was first discovered by Malassez in 1874 who called it ' a spore ' Unna rediscovered it and called it '*faschen bacillus*' or flask bacillus, as some of these are like the shape of a flask Dermatologists nowadays call this by the popular name of bottle bacillus ' as many of them are the shape of a gourd Since the discovery of the bacillus by Malassez about half a century ago, it had never been cultivated successfully up till now, although various attempts were made to solve the difficult problem I have searched the literature on the subject with a negative result Rulison and Highmore of New York in *Archives of Dermatology and Syphilology* Vol X 1924, write thus—' the scale of *pityriasis simplex capitis* always shows large numbers of a special organism—probably an epidermophyton *which has never been* cultivated ' Templeton of Oakland, Calif, in his excellent article on the study of dandruff and of the *Pityrosporon* of Malassez published in the *Archives of Dermatology and Syphilology*, September 1926, says that Sabouraud tried first all the common laboratory media and then special ones, such as, bouillon from human skin, decoction of human hair, gelatin with human urine, egg yolk, all sugars peptone, infusion of grains human urine with potato and gelatine, etc but in spite of all his attempts he failed to grow the organism The great mycologist then wrote the following lines at the conclusion—' After thousands of experiments, one can say that the *Pityrosporon* of Malassez (bottle bacillus) is not cultivated elsewhere than on the cornified epidermis of man '

Templeton himself tried various media, such as Sabouraud's media, at different pH, Sabouraud's medium with oleic acid, brain media, beer wort agar at 6 to 7 pH, Russell s medium, beer wort with 1 per cent oleic acid, egg, litmus, sugars like galactose, lævulose, saccharose, lactose, Avery's medium, and calcium carbonate

medium He tried anærobic cultivation also, but after all these attempts, he too failed to grow the organism He simply suggested that the ' bottle bacilli ' could be grown artificially He says, after three to four days' inoculation, there is merely a slight widening of the area occupied by the dandruff scale—a meagre growth in *one* instance in subculture ' He does not mention the nature of the widened area and the characters of the meagre growth The microscopic photograph of the smear of his subculture is very hazy and shows nothing but degenerated ' bottle bacilli ' from disintegrated scales A doubtful success in one instance alone in subculture out of probably hundreds of experiments is never suggestive of actual success His final argument is that as *bottle bacilli* grow on the scale, he believes, therefore, that ' the *Pityrosporon* of Malassez (*bottle bacillus*) *can* be cultivated successfully ' It is clearly seen, therefore, from his own words that he has not cultivated the organism but hopes to do so in future

Bottle bacilli ' do certainly grow on the dandruff scale as is shown by budding and the presence of large numbers of them in the scale That they grow on the scale when placed in artificial laboratory media was also observed by us long ago on Sabouraud's maltose agar But the two essential points regarding successful culture, namely, the character of the primary growth and a pure subculture in not only one but in a large percentage of cases still remained unsolved The latest report on the ' bottle bacilli ' is to be found in Aldo Castellani's lecture on Fungi and Fungous Diseases, published in the *Archives of Dermatology and Syphilology*, October 1927, where he says that the organisms have not been cultivated as yet Similarly, *Microsporon furfur*, the causative organism of *pityriasis* or *tinea versicolor*, has not been cultivated successfully

My object in writing on both the bottle bacillus ' and the *Micosporon furfur* in one article is to show that both of them have been cultivated by me successfully and both belong to the same genus, the species only being probably different *Microsporon furfur*, as far as is known, was first described by Charles Robin in 1853 i e , about three quarters of a century ago Castellani in 1905 says attempts at cultivation have failed,' ' the fungus does not grow on artificial media ' Sidlick and Corson of Philadelphia in the *Archives of Dermatology and Syphilology*, May 1922 writes thus—' though numerous mycelia and spores typical of *Microsporon furfur* were found—we were not successful in our repeated attempts to cultivate the fungus ' Castellani in his latest article, October 1927, says, ' cultivation has not yet succeeded '

From all these reports, it can safely be concluded that the bottle bacilli ' of dandruff and *Microsporon furfur* of *tinea versicolor* and *flava* have not been successfully cultivated I shall show in this paper that they have been grown for the first time in cent per cent of cases in our laboratory in the Calcutta School of Tropical Medicine

Hitherto, the classification of the above two organisms has been as follows — The ' bottle bacillus ' belongs to the family *Cryptococcaceæ*, described by Kutzing, 1833, and to the genus *Pityrosporon*, created by Sabouraud, 1895 The *Pityrosporon*

means a *cryptococcus* without a well developed contour At that time Sabouraud made out one species and called it *Pityrosporon ovalis* or *Malassezi* Before Sabouraud created the genus *Pityrosporon*, 1895, Bizzozzero in 1882 called this organism, *Saccharomyces ovalis* Castellani, in 1908, added another species and named it *Pityrosporon cantliei*, where the spores were roundish and usually larger

Microsporon furfur belongs to a different family, namely, *Haplographiaceæ*, described by Saccardo 1896, where hyphæ are present Baillon created the genus, *Malassezia* in 1889 Two species are known, *Malassezia furfur*, 1889, and *Malassezia tropica* 1905 Castellani So the 'bottle bacilli' and *Microsporon furfur* belong to different families, as in one no hyphæ and mycelia are present A summary of the classification, hitherto adopted is as follows —

Hypomycetes

Family No I —Cryptococcaceæ, Kutzing, 1833

Genus — Pityrosporon, Sabouraud, 1895, i e, *cryptococcus* without well developed contour no hyphæ present

Species —(i) *Pityrosporon ovalis* or *Malassezi* Sabouraud, 1895—spores oval and small

(ii) *Pityrosporon cantliei*, Castellani, 1908—spores roundish, usually larger

Family No II —Haplographiaceæ, Saccardo, 1896

Genus —Malassezia Baillon 1889—hyphæ present

Species —(i) *Malassezia furfur* 1889

(ii) *Malessezia tropica* 1905 Castellani

From the above classification, it is evident that the genus was first created by Baillon in 1889 who named it *Malassezia*' Sabouraud later in 1895 created the genus *Pityrosporon*' As I have proved that these two genera comprise one class of organisms we must have *one* genus only The point to decide is which of the above names should stand Although the term '*Pityrosporon*' is more suitable having conveyed the meaning of *pityron* or scale and *sporum* or spore, i e spore like bodies being found in scales of dandruff and *tinea versicolor* and *flava*, still the genus '*Malassezia*' being created first, should have preference Hence, the classification should be as follows —

Family —Cryptococcaceæ

Genus —Malassezia

Species —(i) *Malassezia ovalis*—the cause of dandruff, seborrhœa, seborrhœic dermatitis and alopecia

(ii) *Malassezia furfur*—the cause of tinea or pityriasis versicolor

(iii) *Malassezia tropica*—the cause of tinea flava (*chhuli*, Bengalee, *Scula*, *banruff*—Hindi)

Sabouraud's belief that the 'bottle bacilli' belong to blastomycetes is no longer correct as mycelial forms of the above bacilli have been found by McGuire in the scales of dandruff

Definition The genus *Malassezia* includes organisms of yeast like forms which divide by budding and form short tortuous mycelia, either few or numerous and broken into separate segments The segments bear hyphæ which carry round or oval conidia, either solitary or in grape like masses No asci and lateral buds have been found

Attempts at cultivation I have tried to cultivate the bottle bacilli' for the last few years Dry dandruff scales were selected as the inoculum since the bacilli were present in them in large numbers in stages of division As they took a deep stain they were supposed to be alive Moreover other organisms were usually not seen in the scales My systematic attempts began in December 1925 Difficulty in culturing was chiefly due to contaminating organisms like staphylococci sporing bacilli and fungi Although I knew that all laboratory and various special media had been tried by Sabouraud and others and that there was therefore no need to repeat these I still had a mind to try ordinary agar first of all A few scales from a scalp were examined first and numerous dividing forms of the organisms were seen Then fresh scales were scraped off with a sterilized knife treated with absolute alcohol for 15 minutes washed in sterile saline and then planted on agar The following were the results —

1st Experiment

First day—nil
2nd *Staphylococcus albus* and some other bacill
4th
10th

2nd Experiment —Scales steeped in saline alone for 15 minutes as absolute alcohol might have possibly killed the bottle bacilli Results after the first day other bacilli

Later the whole surface of the medium was coated with the same organism

3rd Experiment —Scales treated with 1 per cent carbolized saline for 15 minutes

Results same as above in the second experiment

In all these experiments the results were negative

On the 26th of February 1926 from the sero pustular lesions of a case of pityriasis steatoides of the scalp a culture was made on sheep s blood agar *Staphylococcus aureus* and fine colonies like those of streptococci developed On a smear examination of the fine colonies big spherical and bottle forms as well as streptococcal chains were seen (Fig 1) A subculture from a fine colony was made on blood agar The organism in culture was fine sago grain like, transparent and strongly hæmolytic A fairly good growth was obtained on glycerine agar After four days cultivation the colonies looked like tiny dew drops by reflected light and by transmitted light when examined by a hand lens each colony showed a central raised point with clear crenated margins (Fig 2) As bottle forms were clearly seen I was convinced that these organisms were the actual 'bottle bacilli' A further study was therefore made as to their characters on various media solid and fluid media at different pH, sugar reactions staining properties relation to *staphylococcus aureus* and *albus* effects of temperature and anærobiosis etc In fact much labour and time were spent on this organism They differed from streptococci in three main points—their macroscopic and

microscopic appearances and their sugar reaction glucose in Hiss' serum water, as all streptococci do bottle forms might form under adverse conditions in kept outside the incubator and the media were all and to my surprise more yeast forms were seen so big forms were the resting forms and the cocci th





'bottle bacilli' Along with these experiments I trie organism from a large number of cases of dandruff, try emulsion of fine scales aerobically and anaerobically, I single case Thus, I failed to prove the organism t without, at the same time knowing what it was

Later I got from a case of generalized seborrhoeic d (Fig 3) the colonies of which were pale white and abunc penetrated and produced gas bubbles inside the solid medi experiments were not uniform One striking thing wa

A dermal inoculation was then made on my own skin with the culture and a control was made with known *staphylococcus albus*, but the results were not suggestive All these experiments made me more or less certain that *staphylococcus albus* was not the 'bottle bacillus' in some stage of development The albus in dandruff is probably what is called the morococcus Once I got from the scalp of a case of seborrhœa pleosa a white creamy growth which turned pale orange yellow later The colonies were very sticky and came off with the platinum loop like a piece of thread and gave out a kind of fæcal odour Microscopical examination showed a few bottle forms On several occasions transparent colonies of a cocco bacillus were obtained During all these experiments I was groping in the dark not knowing what a colony of the bottle bacillus' would look like—whether fine or yeast like or downy whether in culture bottle forms would alter to coccal and bacillary forms etc

On the suggestion of Colonel Acton oleic acid agar was tried but no growth was seen excepting cocci Toisson's fluid in our laboratory used to be often contaminated with budding yeasts and hence on the assumption that it might be a suitable medium for the 'bottle bacilli ' I tried it but without any success On the same theory Raulin's medium and Sabouraud s maltose agar at a pH varying from 5 to 10 were tried but to no effect Scales were cultivated in complete anærobiasis on various media but only *S albus aureus* and diphtheroids grew One day a curious incident happened While examining a suspected colony by staining numerous dividing yeast forms along with staphylococci were seen We all saw the slide (Fig 4) and were more or less convinced that those yeast forms were the bottle bacilli ' Some big forms were seen ruptured and cocci were noticed as if coming out from them So we thought that the morococci found in acute seborrhœic dermatitis were spores of the bottle bacilli ' but a few minutes later to our utter surprise I found that the saline with which a film was made contained a large number of budding yeasts From this it was clear that there were many fallacies encountered before the final goal of truth was reached

After all these attempts a period of lull came and fresh attempts at cultivation were made only about eight months back On the suggestion of Colonel Acton that the *staphylococcus albus* and bottle bacillus might be living symbiotically I cultivated the albus on Sabouraud's maltose agar scraped off the growth washed it in sterile saline and then exposed the culture tube in the sun for two hours to kill all the staphylococci The tube was next incubated and no growth of the albus was seen Dandruff scales were then sown but even after several days of incubation no growth of bottle bacilli ' was seen One day on examination of a tiny bit of scale from a culture on Sabouraud s maltose agar it was found that the scale was disintegrated and contained besides staphylococci, a large number of 'bottle bacilli ' (Plate XII fig 5) In fact the whole scale itself did not show as many 'bottle bacilli ' as were seen in the tiny bit after culture It was therefore concluded that the bottle bacilli grew in the scale Hence we tried to prepare some

special media A packetful of scale was collected from a case of psoriasis and a 5 per cent scale agar' medium was prepared but cultivation did not succeed in either aerobic or anaerobic conditions Colonel Acton then suggested the following cystin media —

(1) Cystin salt saccharose and water,
(2) Cystin salt glucose and water
(3) Cystin salt glucose stearic acid and water,
(4) Each of the above three made solid with agar

Each time however staphylococci and fungi spoiled our culture and did not allow us to observe the scale for a sufficiently long time It was then decided to kill the cocci and fungi without killing the 'bottle bacilli' at the same time I may add here that no bottle forms were seen on cultivation of *staphylococcus albus* on the cystin media even for 10 days

Taking for granted that yeasts and 'bottle bacilli' were probably of the same nature I tried the effect of alcohol for 15 minutes and exposure to the sun for 2 hours on albus and yeasts but found them both killed hence alcohol was unsuitable as an inhibitory agent Next I inhibited the albus by putting the scales on plaster of Paris surrounded by moisture but although the staphylococci were inhibited no growth of 'bottle bacilli' was seen Then I tried the effect of gentian violet to kill the staphylococci and fungi Glucose and maltose agars with 0 001 per cent of gentian violet were tried the 'bottle bacilli' were seen multiplying in the scales while the cocci and fungi were inhibited to a certain extent but all subcultures became negative I found later that if the scales were cultured first on glucose agar with gentian violet for two to four days and then a subculture made from a non contaminated scale on glycerine agar very fine colonies of 'bottle bacilli' could be seen At that time however, I expected the 'bottle bacilli' would have creamy white yeast like colonies so that I missed the fine colonies

Leaving the cultivation of the 'bottle bacilli' for a time I then attempted to grow *Microsporon furfur* of tinea versicolor as I had observed some similarity between the two clinically and on microscopic examination of the scales I found them growing distinctly on maltose agar in the scales along with staphylococci Mycelial forms were no longer seen and numerous deeply staining budding forms were present A bit of the scale so planted was taken up washed in sterile saline and then smashed and made into a fine emulsion A plate culture was made on maltose agar several times but no microsporon was to be found but only staphylococci I therefore tried an albuminous medium like egg with a little glycerine and gentian violet as it was found that the staphylococci grow feebly on the albuminous material with gentian violet

Accordingly a medium was prepared following Petroff s formula namely meat infusion in 15 per cent glycerinated water and the whole content of an egg in equal parts but I modified the amount of gentian violet by adding 0 001 per cent instead of 0 001 per cent Scales from cases of dandruff and tinea versicolor were collected with aseptic precautions soaked in saline and then planted on separate tubes over

moist areas in the lower part as well as dry areas in the upper part of the slants The following are the results of the experiments —

1st day after culture—nothing visible, no contamination

2nd „ „ „ „ „ „ „

3rd „ „ „ —aspergillus beginning to appear , here and there fine chalky growths being visible on a few non contaminated scales of tinea versicolor at the dry part of the slant A small bit of the chalky growth was picked up and examined under a microscope and to my great surprise a large number of typical bottle forms of organisms like spores in tinea versicolor were seen A subculture was at once made on the same medium and in 2 to 3 days' time chalky bead like growths were seen at the junction of the dry and moist parts of the medium and pinkish bead like masses above and below the junction A second subculture was made separately on glucose glycerine and maltose agar and a pure culture of fine, slightly crenated colonies was obtained in 2 to 4 days Microscopical examination showed nothing but bottle and yeast forms

The tubes in which the scales of dandruff were planted were spoiled due to a heavy contamination with fungi So clean scalps were tried again and pinkish growth in some, faintly chalky in others or both types from the same scalp were obtained During cultivation of the ringworm fungi chalky growths are some times found and so there was some doubt as to whether the chalky culture got by me was really the growth of 'bottle bacilli' I then tried cultivation of scales from normal skins, but got negative results A few days later the chalky growth from the scales of tinea versicolor showed on examination the typical jointed mycelia of *Microsporon furfur* (Plate XII fig 6) proving thereby that the growth was a real one This was further corroborated when typical short tortuous segmented mycelia were seen projecting out of some of the colonies on glycerine, glucose and maltose agar tubes (Plate XII fig 7) The successful cultivation of 'bottle bacilli' and *Microsporon furfur* was thus finally solved

Experiments were now made to find out the best medium for primary culture and the nature and least amount of inhibitory agent necessary for checking contamination Subcultures were therefore made on all laboratory media, a few cultures were kept in complete anaerobiasis, different strengths of gentian violet and crystal violet as inhibitory agents were tried and finally a medium consisting of equal parts of egg and meat infusion in 15 per cent glycerinated water tinged with 0 001 per cent gentian violet was found to be the best for primary culture Egg was proved to be the most suitable food for the organisms and gentian violet the suitable antiseptic and befitting background for the easy detection of the malassezia The following are the experiments —

1 Subculture on glucose-agar—best growth in 2 to 4 days time On glycerine-agar—good on maltose agar—good, on modified Petroff s medium with 0 004 per cent gentian violet—good

2 Glucose agar was found to be the best but primary cultures made on glucose-agar with gentian violet 0·004 per cent showed very scanty non chalky growth, while staphylococci not completely inhibited Subculture from this scanty growth on modified Petroff s—growth was good not along with staphylococci Thus glucose agar was not suitable for primary cultivation

3 Subculture on glucose agar with gentian violet 0 004 per cent—*negative even after 10 days*, showing again that glucose with gentian violet was not suitable for primary culture from scales

4 Subculture on glucose agar in partial anærobiasis—good growth Incomplete anærobiasis very slight growth after six days The same culture was kept ærobically for two days—very good growth Showing that the bottle bacilli and *Microsporon furfur* are ærobic but that complete anærobiasis for 6 days does not kill them Similarly, glycerine and maltose agar were tried with the same results *Conclusion* primary culture ærobically on the modified Petroff's medium is the best and *secondary cultures are equally good on glucose, maltose, glycerine, Petroff's medium*

5 *Petroff's medium with gentian violet, 0 002 per cent*

(i) Primary culture—greater contamination

Secondary culture—better and quicker growth of the malassezia So 0 004 per cent of gentian violet hinders the growth not only of fungi and staphylococci, but also of malassezia to some extent

(ii) *Petroff's medium with gentian violet, 0 01 per cent*

Primary—scanty growth

Secondary from the primary —nil Therefore 0 01 per cent is too strong and sometimes kills the malassezia The object of this experiment with higher strengths of gentian violet was to inhibit the *contaminaters completely and then make a subculture on glucose* agar to get the pure growth

6 *Petroff's medium with crystal violet, 0 001 per cent*

(i) Primary—nil and sometimes positive

Secondary—positive

(ii) *Petroff's medium with crystal violet* 0 004 per cent

Primary positive, non chalky and not so well seen as against gentian violet background

Secondary strongly positive, but less so as in Petroff's with gentian violet Non chalky and hence less visible

Conclusion —Gentian violet, 0 004 per cent is less toxic than crystal violet of same strength Moreover, primary cultures may become chalky and the background with gentian violet being better, isolation becomes easier

7 *Experiment to find out which of the constituents in Petroff's is suitable for the malassezia* —

(i) *Contents of an egg with gentian violet, 0 004 per cent*—primary culture is positive This is important, as it shows that egg is essential for primary cultivation The growth is very slow and the colour of the background is not satisfactory The only advantage is that contaminating fungi and cocci are absent or less in numbers

(ii) *Meat infusion in 15 per cent glycerine, agar and gentian violet, 0 004 per cent with slightly alkaline reaction*—Primary as well as secondary growths very feeble, sometimes absent entirely

(iii) *Half part of meat infusion in 15 per cent glycerine and one part of egg with gentian violet, 0 004 per cent* Primary—growth poor or nil, less chalky and fungi *abundant* Secondary—poor growth

Conclusion Meat infusion and egg are both necessary for the best cultivation of the malassezia

Technique of primary cultivation of the Malassezia Scales are collected by scraping on the edge of a sterilized knife and then transferred to a saline solution in a watch glass They may also be collected between two sterilized slides and may be used for cultivation later After a few minutes the scales are taken out from the saline and planted in the dry upper part of the culture tube as already described. About 6 points are inoculated and at least two tubes are used Soaking the scales in saline is not always necessary, but I find that one big scale, soaked, may be easily divided by the loop in the tube and planted at 6 different points The culture is incubated at 37° C and examined day after day for early signs of chalky or pinkish bead like growths It is essential that the tubes should be examined every day,

preferably with a hand lens otherwise fungi may contaminate the cultures If any fungus be seen early it should be killed with a heated rod and if any growth of the malassezia be visible it should be transferred at once to get it pure As a rule in 1 to 3 days time the growth becomes visible as small chalky points on the scales or pinkish bead like masses (Plate XIV fig 8) A subculture should be made one on the modified Petroff s medium and another on glucose agar The colonies on glucose agar are seen in 1 to 3 days time as minute crenated points best observed by transmitted light (Plates XIV and XII figs 9 and 10) If the culture is pure then it is kept in the incubator for 2 to 4 weeks in order to study the further changes in its appearance

Cultural characters Two types of cultures are seen One is chalky and the other pinkish The chalky character develops best at the junction of dry and moist areas of the medium (Plate XIV fig 11) and is not seen in any other media excepting modified Petroff s Both chalky and pinkish characters may be seen in one and the same type of culture (Plate XIV fig 12) When examined by a hand lens each colony is seen to consist of a bead like mass which gradually enlarges by fine concentric rings A chalky colour develops on the top of the colony and when several colonies fuse together a fossil like mass forms Some of the colonies become elongated like a pyramid with a ring at the base and flattening at the top (Fig 13) Sometimes the chalky colour fades away later Finally, after about 2 months a faint orange colour is seen on the surface of a chalky culture (Plate XIV fig 14)

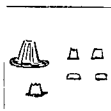


Fig 13
Colonies of Malassezia

The pinkish type of growth too is seen to consist of two or more bead like masses surrounding which a fine surface growth extends into the medium After one to two months an orange colour develops on the surface (Plate XIV fig 15) As a rule *Malassezia furfur* is chalky and *M tropica* is pinkish The former causes a brown or greyish corrugated lesion on the neck chest axilla and fore arms and the disease is called tinea versicolor The latter is more commonly met with here It attacks the face around the mouth the neck chest back and sometimes the palms and gives rise to yellowish white depigmented patches which are so characteristic of the disease Sometimes both types are seen in one and the same patient *Malassezia ovalis* also shows two types of growth as above on the egg medium

Considering the facts that a chalky growth can be changed into a non chalky form and vice versa it is doubtful whether two types of growth do really exist

Subcultures The organisms grow on all laboratory media in subculture best in glucose and glycerine agar In fluid media the growth is scanty due probably to the fact that it is hindered by too much moisture No growth takes place on blood agar serum and potato This may explain the fact that these organisms rarely penetrate the deeper layers of the skin

Colonies on the culture media present a typical crenated appearance Three types are commonly seen the first is crenated with irregular spiky projections here and there (Plate XII figs 7 and 16) the second is like a star with distinct radial arrangement and a thick mass of spores in the centre (Plate XIII fig 17) and the third is like a piece of cauliflower or coral branching variously (Plate XIII fig 18) Under the microscope each colony is seen to consist of a large number of budding forms some being grouped in masses If cultures of *Malassezia furfur* and *tropica* be kept for 7 to 10 days typical tortuous jointed mycelia are seen to develop out of some of the colonies (Plate XIII fig 19) This is best seen by *examining the culture tube with a* ⅔ *lens under the microscope* Later grape like masses of conidia are seen and mycelial forms disappear largely

The malassezia grow also outside the incubator and live long Hence a sub culture once a month is sufficient They do not grow in complete anærobiasis but they are not strict ærobes

Culture at different pH The organisms grow at a pH varying from 5 to 9 The best growth is seen at a pH of 5 to 7 5 The colonies although less numerous *are bigger and more discrete At a pH of 8 to 9 they are minute less distinct and* more numerous Mycelial forms are seen best at pH 6 and they seem to be less above pH 7

Sugar reactions—No sugars are fermented

Relationship with staphylococcus albus *Staphylococcus albus* inhibits the growth of the malassezia This is proved by the following experiment A glucose agar tube is inoculated with *staphylococcus albus* on the lower half of the slant *After 24 hours culture the growth is scraped off and washed away with saline* The tube is then exposed to the sun for 2 hours and then incubated for 24 hours No growth of albus is seen after incubation showing thereby that exposure to the sun for 2 hours kills them The whole slant is now inoculated with malassezia and after about 3 days colonies of malassezia become visible on the upper half of the slant where there was no albus but on the lower half where the albus was grown a few colonies of the malassezia are seen showing thereby that *staphylococcus albus* renders the soil unsuitable for their growth Moreover albus and malassezia have been mixed together and grown and the result has been the growth of albus almost alone

Morphology

Malassezia ovalis The organism as described by Sabouraud is polymorphous We have found in the scales spherical, oval coccal and occasionally mycelial forms In culture single and budding forms alone are seen (Plate XIII fig 20) Short mycelial forms are sometimes seen but no typical long mycelial and morococcal forms have as yet been observed although cultures have been examined repeatedly in a course of three months Fig 21 shows some stages of development Big oval forms have *their budding surface usually* plano concave and the bud seems to emerge from the interior of the mother cell

Sometimes two small elongated buds like short mycelia are seen Later when the mother cell dies, it looks like a broken empty egg shell

Malassezia furfur and tropica The organisms show the same morphological characters as above excepting that, sooner or later mycelial forms develop Usually two mycelia grow out from one mother cell (Fig 22) Sometimes the mother cell itself elongates and we find three mycelial rods meeting at one point These gradually become elongated, tortuous and segmented (Plate XIII, fig 23) This is best seen on the dried part of solid media at a pH of 6 Later, mycelial forms tend

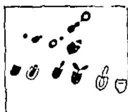


Fig 21
Bottle bacilli

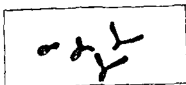


Fig 22
Malassezia furfur & tropica

to disappear and here and there attached to mycelia groups of conidia in grape like masses are seen Irregularity in contour of the mycelia as has been observed by Castellani in *Malassezia tropica* has not been seen by me as a constant and characteristic feature

Staining reactions The malassezia stain well with all aniline dyes They are Gram positive and non acid fast

Immunity reactions It is difficult to kill the malassezia completely, especially the 'bottle bacilli' by drugs like resorcin sulphur, iodine and hydrarg perchlor Hence, it is desirable that the soil in which they grow should be rendered unsuitable for their growth Thus a stock vaccine of bottle bacilli has been prepared and is being tried in our out patients' department in cases of deep types of seborrhoeic dermatitis It is difficult to pass any definite opinion at present Hopeful results are being observed in some cases

Action of antiseptics Formalin vapour does not kill the malassezia in two hours although the fungi of all ringworms are killed in one hour All the malassezia are killed by sulphur dioxide in half a minute or it may be in less than half a minute It is probably for this reason therefore, that sulphur is so valuable in seborrhoeic dermatitis

Inoculation experiment to prove Koch's postulates This experiment has been done with some measures of success It is difficult to get normal scalps without 'bottle bacilli' One must also have susceptible skins and a suitable season for inoculation experiments The experiment has, therefore, as yet not been given a fair trial

My thanks are due to Colonel Acton for kindly helping me with his valuable suggestions

Explanation of Plate XII.

Fig. 5 Growth of 'bottle bacilli' and staphylococci
" 6 Smear from culture of *M. furfur*
" 7 Colonies of *M. furfur* showing mycelia
" 10 Colonies of 'bottle bacilli' (*M. ovalis*)
" 16 Colonies of *M. furfur*.

PLATE XII

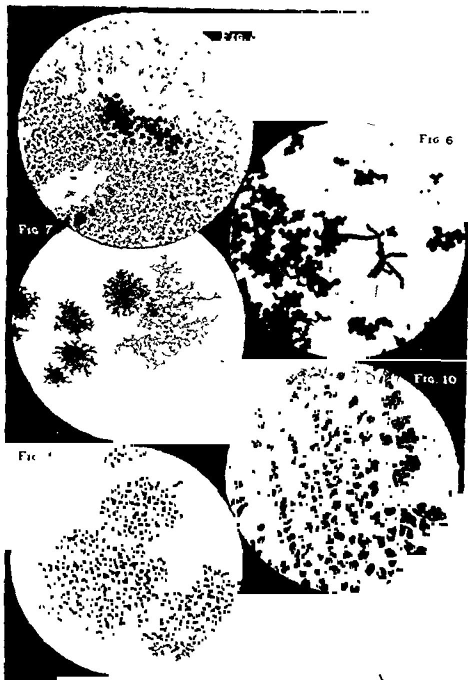


PLATE XIII



Fig 20



Fig 19

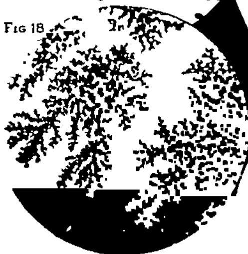


Fig 18



EXPLANATION OF PLATE XIII

Fig 17 Colony of *M furfur*
„ 18 Colonies of *M ovalis*
„ 19 *Malassezia tropica.*
20 Smear from culture of ' bottle bacilli '
, 23 *Malassezia tropica*

Explanation of Plate XIV.

Fig	8	*Malassezia furfur*, Primary.
„	9	„ „ Secondary. (Small figure, same magnified.)
„	8a	*Malassezia ovalis*, Primary.
„	9a	„ „ Secondary. (Small figure, same magnified.)
„	11	*M. ovalis.*
„	12	'Bottle bacilli.'
„	14	*M. furfur.*
„	15	*M. tropica.*



Fig 8



Fig 9

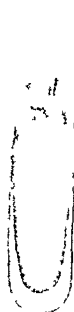


Fig 8(a)

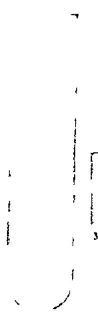


Fig 9(a)

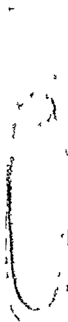


Fig 11

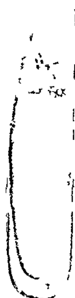


Fig 12



Fig 14

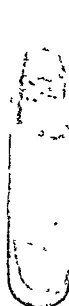


Fig 15

THE STREPTOCOCCI AND THEIR IMPORTANCE IN THE TREATMENT OF TROPICAL DISEASES

BY

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History

This very important member of the biological group micrococcaca has been known to both physicians and surgeons since it was first described by Pasteur and Doleris for the tissue changes that they produce by causing induration and production of toxins which cause destruction of the red blood cells fever and other general toxic symptoms The study of the individual members of this vast family which consists of no less then 40 members was not commenced till 1909 10 by Rosenau and later on important discoveries as to the nature of the lesions produced and the toxins elaborated in the tissues were made from time to time by later observers

It is an interesting fact that a practitioner in the tropics hardly ever comes across cases of rheumatic fever or scarlatina Chorea is a disease practically unknown in this country and the incidence of this disease has not been referred to by clinicians either in hospitals or private practice

During the six years work in the skin out patient department attached to the School of Tropical Medicine only four cases of purpura have been recorded and this goes to prove that this particular lesion of the skin is fairly uncommon in the tropics Although there is a good deal of controversy about the streptococci being actually the cause of these three common diseases of temperate climates the relationship of the clinical manifestations of rheumatic fever scarlet fever and purpura with streptococcal infection is a true one It is difficult to state definitely whether these particular types of streptococci do not exist in the tropics at all the probabilities are that the nature and biological characters of these varieties may have been altered owing to changes in the environment and the susceptibility of the individuals affected As the virulence of streptococci varies with the nature and type of the other symbiotic organisms the rarity of rheumatic and scarlet fevers as well as of purpura may also be due to the fact that the normal oral and nasopharyngeal flora are widely different in tropical and temperate climates It is not possible at present to prove this latter statement by experimental facts, but considering that Rogers and Vincent have successfully enhanced the virulence of streptococci to laboratory animals by inoculating them with old avirulent strains along with dead cultures of *B proteus vulgaris* and *B typhosus* the latter theory may be taken as a sort of working hypothesis without running the risk of grave error The statistical figures of the general and maternity

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hospitals in India show that empyema and puerperal septicæmia due to streptococci are very nearly the same as in temperate climates, this may be due to the fact (as already stated) that the symbiotic organisms in these two conditions are very much the same in tropical and temperate climates

I Lesions on the Surface of the Skin

(1) *Primary infections*

Practically all the streptococci that have been isolated so far in the pathological laboratory, are from the skin clinic attached to the Tropical School As primary lesions two varieties of impetigo have been met with, viz, the superficial and the deep types

(*a*) In the *superficial type* the classical impetigo contagiosa the lesions are all multiple with a dry looking yellowish scab, they are fairly infective and in healing leave no scars The isolation of streptococci is fairly easy, so long as there is no staphylococcal infection along with it The surface is cleaned with a little alcohol and the primary culture taken on blood agar from near the edge of the lesion The streptococci are all hæmolytic and appear as very fine colonies on blood agar Primary cultures on other media are not so successful The types isolated from these lesions are cutis 1 and 2

(*b*) The *deep type* of impetigo resembles Veldt sore There is a good deal of induration and there is more tissue destruction as compared with an ordinary impetigo In healing this type always leaves a good deal of scarring of the skin The streptococci obtained from this type are quite distinct from those isolated from the superficial type although the sugar reactions of these two varieties are the same in most cases The colonies are hæmolytic, larger, dry looking, rather difficult to pick up with the platinum loop, and do not emulsify easily The streptococci isolated from both of these lesions belong to the Beta type of Brown, forming fairly long chains in glucose broth This variety of impetigo is fairly resistant to ordinary local treatment by hydrarg ammon ointment, and does not clear up unless an autogenous vaccine is given

(2) *Secondary infections*

Superficial lesions —Of all the skin diseases that have been treated at the out door clinic nearly 60 per cent were secondary streptococcal infections implanted on either tinea or seborrhœic dermatitis The true nature of the lesions is often masked by the induration and oozing of serum which is the result of the secondary infection In cases of tinea, what happens is that the mycelia open out the intercellular spaces in the prickle cell layer of the skin and the breach in the surface horny layer allows the streptococci to gain entrance into the lymphatic stream As streptococci and staphylococci are the only two organisms that grow in serum, the clinical picture is that of an ordinary superficial streptococcal dermatitis, as long as there is no secondary staphylococcal infection The streptococci are easily obtained by taking cultures from the oozing serum on blood agar In cases of seborrhœic dermatitis

the irritation causes a condition of lymphatic turgescence underneath the horny layer, and when the surface is broken by scratching, the secondary infective organisms gain an entrance The true nature of these lesions is not apparent, till all this induration and oozing has been thoroughly treated by suitable cooling and evaporating lotions like lotio calamine, etc In all these cases the local symptoms are most prominent, and the general symptoms are only caused by irritation, sleeplessness, etc In healing these do not leave any scars, but the infected area appears a little glazed and pigmented The types of streptococci isolated are as follows —Cutis 1 and cutis 2, hæmolyticus 1 and hæmolyticus 3

II IN THE SUBCUTICULAR AND DEEP TISSUE

(1) *Lymphangitis* —Most of the cases analysed were confirmed cases of previous filarial infection There was a good deal of local œdema, induration, swelling, pain and tenderness, and nearly always accompanied by fairly high temperature The causative organism was more difficult to find in these cases and in comparatively rare instances when a breach in the surface appeared, the streptococci were cultivated from the exuding fluid Repeated attacks of this kind lead to permanent swelling owing to fibrosis of the soft subcutaneous tissue

(2) In other instances, the local manifestation is not so prominent but there is very high fever sometimes accompanied by delirium, the only local manifestation in such types of cases being an erythematous rash either confined to one limb or distributed to different parts of the body accompanied by much joint pain Such a case coming under observation for the first time may easily be confused with one of acute exanthemata, and the diagnosis is not established until the temperature comes down with rest in bed in about three to four days The local manifestations take about a week to ten days to subside There is practically no staining of the skin after the healing of this kind of rash, and the desquamation is very fine Sometimes there is a periodic exacerbation of these symptoms but the blood culture during the febrile stage has been so far negative A complete course of injections with a mixed streptococcal and staphylococcal vaccine has been given successfully to prevent relapses

(3) *Deep abscesses* in the groin accompanied by high fever and without any apparent cause are sometimes met with A case very often resembles acute bubonic plague if the patient comes under observation in an endemic area during the plague season These deep abscesses may appear in other parts of the body, legs, arms and buttocks, *etc* On opening these abscesses, the pus is usually very thick, culture yields long chain hæmolytic streptococci The types isolated are —

Cutis 1 and cutis 2 mostly, and hæmolyticus 2

III IN THE INTESTINAL TRACT

(*a*) *Pyorrhœa alveolaris* has been known to be the cause of slight minor ailments like dyspepsia, indigestion, and as a chronic condition gives rise to multiple rheumatoid arthritis

(*b*) *Subacute follicular tonsillitis* can in some cases produce neuritis of the larger nerve trunks, like the brachial plexus, and septic emboli deposited in the vicinity of the larger joints, like the shoulder and the knee, have been known to cause paralytic symptoms Treatment of the septic foci relieves the neuritic and joints pains In selecting the causative streptococci, one has *sometimes* got to differentiate the parasitic from the saprophytic types Although Petruschky holds that the same strain of streptococcus can produce such different varied clinical conditions as erysipelas, suppuration and septicæmia (this view has been supported by Horder and Besredka) it has often been found that streptococci isolated from one suffering from early pyorrhœa and no secondary symptoms will have no effect in relieving the symptoms of another whose neuritis and arthritis are the direct result of long standing pyorrhœa or subacute tonsillitis

(*c*) *Gastric or duodenal ulcers* —A few cases have come under observation where the patient has been harbouring a gastric or duodenal ulcer for quite a long time, and the only clinical manifestation was an occasional pain in the region of the stomach and slight indigestion These types of patients carry on their normal work and the only subjective symptoms are a sense of weakness and a general run down feeling In the presence of a secondary infection with hæmolytic streptococci, grave symptoms of anæmia are produced, the condition of the patient steadily becomes worse in spite of hæmatinic and alterative treatment and a fairly well advanced case may clinically resemble cancer of the stomach or duodenum Induration produced by streptococci on the musculature of the stomach hinders the normal peristaltic movements, and a skiagram taken after a bismuth meal often shows distinct evidence at the site of the ulcer The causative streptococci can only be recovered after repeated bacteriological examinations of the stools, the hæmolytic streptococci may be found after seven to eight samples have been plated on suitable culture media In the Tropical School pathological laboratory, the best medium for the favourable growth of streptococci has been found to be Conradi's medium, in which the lactose has been replaced by glucose A few cases have been very successfully treated with auto vaccines prepared from hæmolytic streptococci, obtained from the stools of such patients

(*d*) *Dysenteric ulcers* —The chronicity of dysenteric ulcers is maintained in most cases by secondary infection with the intestinal type of streptococci alone or along with other non sporing ærobes Ulcerations produced by the dysentery bacilli may either cause intestinal stasis by blocking the normal peristaltic movements at the site of the lesion, or intense diarrhœa owing to the hinderance of absorption from the surface of the mucous membrane of the intestines In cases of intestinal stasis, the secondary infective process has got a better chance of hindering the healing of the ulcers In some cases the secondary infective organisms completely overgrow the original dysenteric bacilli and repeated bacterial cultures may fail to isolate the latter In these cases the clinical picture may resemble one of sprue or tubercular enteritis. To establish our diagnosis, it is necessary to examine the agglutination reactions of the patient's blood against organisms of the dysenteric

group When hæmolytic streptococci are the predominant secondary organisms, grave symptoms of anæmia with or without fever may be produced In isolating this particular strain of streptococcus, repeated plating of the stools on glucose Conradi's medium may be necessary In other instances septic emboli containing streptococci may be carried away from the site of the lesion into other parts of the body and be deposited near the larger nerve trunks like the sciatic or they may produce inflammatory reactions in the hip and sacro iliac joints Consequently, the entire picture is altered into one of arthritis or neuritis and the subjective symptoms do not point to intestinal causes at all The treatment of these cases is sometimes rendered more difficult by the fact that the fæcal type of streptococci, which is sometimes present in an apparently healthy subject, can produce these lesions under favourable circumstances, and in our bacteriological culture we very often tend to overlook the fæcal streptococcus as being a non toxic saprophyte In rarer instances, where the general condition of the patient is very much run down, metastatic abscesses may be produced on other parts of the body Generalized septicæmia has not come under our observation

As regards amœbic dysentery, chronicity and persistence of histolytica cysts in the stools is most often the direct result of secondary streptococcal infection on the intestinal ulcers Failure of emetine in such cases is explained by Col Acton as follows 'Emetine is mostly excreted through the large intestines During excretion emetine has a direct paralysing action on the amœba, but it is practically inert in strongly acid substrates Whenever there is infection with streptococci—and they grow best on slightly acid media—the tissue reactions are changed into acid, and in this way secondary streptococcal infection on amœbic ulcers hinders the emetine from exerting its specific affect' The only way to treat such patients successfully is to plate out their samples of stool from day to day until the hæmolytic streptococci are obtained. The course of emetine is given after about eight doses of an autogenous vaccine prepared from the stool streptococci An interesting fact has been worked out by our former clinical pathologist, Dr A K Dutta Gupta, when he examined samples of stools from a large number of histolytica 'carriers' mostly amongst the menial staff of the school, who complained of no subjective symptoms Although the microscopic examination showed the typical histolytica cysts in practically every case, bacteriological examinations never gave any hæmolytic streptococci From these data we are inclined to think that the more important symptoms like anæmia constipation and so called dyspepsia of the chronic amœbic carriers are more due to this secondary infection of the large intestine than to the presence of the *Entamœba histolytica* cysts amongst the folds of the mucous membrane

IV In the Urine

The methods of isolating the streptococci from the urine particularly when they are present in very small numbers have been so far very unsatisfactory In our conjoint paper on the 'Causation of Cystitis' in collaboration with

Dr G Panja(1927) we have described in detail the various methods which were adopted in the laboratory for isolating pure cultures The best and most satisfactory method we have found is to put into the incubator about 15 to 20 c cs of a catheter specimen of urine aseptically collected, and after 24 hours the streptococcal colonies appear as small dots or like tiny wisps of down Sometimes they appear as a long comet-shaped wisp like growth These individual colonies are carefully picked up with a sterile capillary pipette and subcultured on blood agar In this case the sterile specimen of urine acts as the enrichment medium and probably the growth of streptococci starts on small molecules of nitrogenous elements like creatin and creatinin present in the normal urine and which act as the nidus for the chains to form The first growth is best helped by the slightly acid reaction of the normal urine and later on the excessive acid production from the growth of the streptococci themselves is checked by the free ammonia present in the urine

The cases that were first studied particularly were those of epidemic dropsy, and later on several cases of pernicious anæmia of unknown origin were studied in the same way The ways in which streptococci and other intestinal organisms may find their way into the bladder are —

(1) *Breaches in the surface of the mucous membrane*—Col Acton, working on the subject of epidemic dropsy, has collected a very valuable series of pathological sections of various organs and from the study of these specimens it can easily be *concluded that intestinal types of organisms can in a condition like epidemic dropsy,* get into the circulation owing to breaches in the surface of the mucous membrane caused by diarrhœa and rapid desquamation of the surface epithelium In all probability both in epidemic dropsy and in the so called idiopathic pernicious anæmia a primary condition of diarrhœa is the fore runner of the infection and the bladder is secondarily *infected,* although this condition can hardly be *termed true* cystitis These organisms first gain access to the blood and lymphatic spaces in the sub epithelial tissues of the intestines and may be washed away into the general blood circulation Blood cultures done on these types of cases have so far yielded negative results The explanation of this negative finding can be given satisfactorily when we remember the fact that the streptococci do not multiply in the peripheral blood which is always of an alkaline reaction and is therefore, not suitable for the streptococci The invasion of the other organs of the body from the original nidus takes place in the form of small embolic showers washed off from time to time Although these minute emboli have got to travel via the blood stream they are so much diluted up by the total volume of blood that it becomes practically impossible to pick them up in 2 or 3 c cs of blood drawn from a vein of the patient except by chance

(2) *Lowered internal defence mechanism* which may be the direct result of long protracted illness or confinement It has been shown by Col Acton and Major Chopra that whenever the internal defence mechanism is lowered there is always a consequent increase in the tissue permeability The potential spaces between the

delicate endothelial cells are widened allowing first an increased flow of serum along with bacteria and later on leucorrhexis and erythrorrhexis Only a few cases of post febrile anæmia of the pernicious type have been studied and the types of streptococci isolated in all these cases are of the intestinal group namely *mitis salvarius* and sometimes *staphylococcus mollis*, which in its behaviour closely resembles the streptococci On blood agar they are faintly hæmolytic forming very short chains They are comparatively more delicate than the other strepto cocci which have been isolated from the skin teeth etc and they die by the time the fifth and sixth subculture is made Besson commenting on this non viability of streptococci advises keeping the culture under anærobic conditions as the strains that he isolated usually died within a fortnight Sometimes cultivation in serum broth or on human blood agar may be useful to keep them alive as laboratory stock cultures The virulence of all these streptococci is very soon destroyed and a fairly heavy dose given to an experimental animal does not lead to death A few cases of pernicious anæmia have been treated very successfully in the Carmichael Hospital and the success of the treatment of these idiopathic types with the autogenous streptococcal vaccine obtained from the urine depends on the preparation of the vaccine immediately after the streptococci have been isolated in pure form

Cases of pregnancy anæmia Only 2 cases were examined by courtesy of Lieut Col V B Green Armytage and Major P Fleming Gow One of these showed the same type of streptococcus both in the urine and stool

The next question that arises in this connection is Is the normal glomerular epithelium of the kidney permeable to bacteria ? A few experiments were carried out in the pharmacological laboratory with the help of Capt Venkatachalam and Dr J C Gupta on the kidneys of anæsthetized cats after the brain and spinal cord had been destroyed and artificial respiration started The kidneys are rapidly dissected out and perfused by sterile normal saline through the renal artery The circulation in the kidney is maintained by means of a Higginson s syringe and the saline is returned by the renal vein The ureter is carefully dissected out and the secretion allowed to flow through the ureter The success of this experiment depends on putting the perfusing cannula in the renal artery before the blood in the finer terminal capillaries in the kidney has time to clot The object of this experiment was to find out whether fresh young [illegible]

the flow from the ureter young broth cultures of typhoid bacilli were selected for injection into this perfusing fluid The injection was given at a certain time and cultures were taken both from the flow from the vein and from the ureter at intervals of three, five, seven and ten minutes All this time the pressure of the perfusing fluid was kept near about 70 mm of mercury After 24 hours' incubation *Bacillus typhosus* was obtained from the tubes inoculated with the return flow in the renal veins but none could be isolated from the flow from the ureter The

experiment was repeated after the pressure of the perfusing fluid was raised moderately high but no bacilli could be isolated from the flow from the ureter The conclusion is therefore that normal healthy glomerular epithelium is impermeable to bacteria

Experiments on the same lines were carried out after the animals had been previously injected with about 25 milligrams of choleramine and beri beri rice base respectively but at this stage further work on this subject had to be postponed for the time being

CONCLUSIONS

(1) Streptococci met with in the tropics are somewhat different from those found in temperate climates Rheumatic fever scarlet fever and purpura are practically unknown in the tropics

(2) As a secondary infection in two of the most common tropical skin diseases—tinea and seborrhœic dermatitis—they play a very important part in masking the true nature of the lesion The intractable nature of most of the so called eczemas is due to this infection

(3) It is the most important organism complicating filarial infection and is the causative agent of most of the clinical manifestations of filariasis

(4) Affections of nerves and joints from a nidus of infection in the teeth throat etc occurs by small embolic showers and grave anæmia is caused by toxic absorption from secondary streptococcal infection in gastric duodenal or intestinal ulcers Negative blood cultures are due to these small embolic showers being missed in the 2 to 5 c cs of blood taken from the patient

(5) Chronicity of bacillary dysentery is largely due to streptococcal infection of the ulcers long standing cases go on to sprue or resemble tubercular enteritis

(6) Failure of emetine to clear the large intestine of *Entamœba histolytica* cysts is mainly due to streptococcal infection altering the tissue reactions at the site of the ulcer into acid emetine being inert in an acid substrate

(7) Many of the severe anæmias of unknown origin are due to hæmolytic streptococcal infection as shown by isolation of the organisms in the urine and clearing up of the symptoms after auto vaccine therapy with the urine streptococci

(8) Normal glomerular epithelium of the kidney is impermeable to bacteria

I desire to express my gratitude to Col H W Acton for the invaluable advice and guidance received from him in getting up this paper I cannot thank him enough for all the trouble he had taken in planning the experiments and interpreting the results

REFERENCE

BANNERJEE K and PANJA G (19[illegible]7) *Ind Med Gaz*

SUR LE COMMENSALISME DE LA FAUNE SPIROCHÉTIQUE DANS LES ARCADES DENTAIRES ET DANS L'INTESTIN DE L'HOMME ET DES ANIMAUX

PAR

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INTRODUCTION

La présente communication a pour but montrer, en me secourant des données fournies par la pathologie comparée, que la faune spirochétienne que l'on rencontre dans le tube digestif humain trouve son homologue chez plusieurs espèces animales. Loin de moi l'idée de déduire de ce fait des conclusions ou des hypothèses tendantes à faire pencher d'un côté ou de l'autre la question si obscure de la pathogénicité ou du commensalisme de ces spirochètes. *A priori* par une similitude logiquement acceptable, il ne serait pas hors de place remarquer que les mêmes problèmes qui se rattachent à tous les commensaux du tube digestif se posent aussi lorsqu'il s'agit des spirochètes: en effet les dégrés de saprophytisme et virulence des cocci pyogenes, de bactéries ou de levures ayant leur habitat normal dans les cavités buccales ou intestinales sont à la merci de facteurs variés dont la génèse souvent nous échappe, étant en général encadrée dans des lois trop réelles mais par trop vagues de la pathologie générale, soient la virulence du microbe augmentée ou la résistence du terrain amoindrie.

Mais ce que je désire faire bien ressortir de ces études c'est qu'on ne doit pas se fier en me limitant tout simplement au problème des spirochètes du tube digestif, cela va sans dire, à la constatation de ces agents pour conclure à l'étiologie spirochétienne d'un état morbide gastro intestinal, vu que l'existence de tels spirochètes montre dans l'espèce humaine ou chez les animaux des variations sans compte, depuis l'absence complète jusqu'à une richesse extraordinaire, indépendantes le plus souvent de tout état morbide appréciable.

Ainsi comme il arrive avec les autres microbes, les bons *à tout faire* de Paul Courmont, je ne doute point que ces spirochètes commensaux puissent entretenir ou contribuent à créer certains états morbides, au même point que les bactéries siegeant dans la bouche ou dans l'intestin. Mais de là jusqu'à la création d'entités cliniques ou anatomopathologiques fondées tout au plus sur le *post hoc ergo propter hoc* le saut est trop grand pour que je ne vienne pas appeler l'attention sur les faits que je signalerai plus loin.

Soit dans la protistologie, soit dans la pathogénèse de ces spirochetes, nombre de points sont obscurs et pas encore resolus D'abord la nomenclature et l'identification des especes ! On peut dire qu'au moment actuel le *microscopiste qui voudrait*, d'apres les descriptions des auteurs, faire une diagnose exacte d'un de ces organismes trouverait difficilement des elements pour arriver à son but L'isolement de ces spirochetes en culture pure dont dernierement le prof Sanarelli nous a donne un très interessant rapport(1) pourra jusqu'à certain point resoudre le probleme surtout lorsque accompagne d'epreuves d'inoculation et d'immunologie croisée Il faudra certainement s'assurer que dans le cas de plusieurs especes avec un habitat commun, la culture appartient a une telle ou telle espece et ne contient pas des individus de toutes les especes ensemble Mais jusqu'à ce que ces méthodes soient entrees dans la pratique courante, dument controlées et perfectionnées, nous devons nous contenter de données morphologiques a l'aide desquelles on peut, en y apportant une caractérization plus rigoureuse que celle que l'on trouve communément dans les livres, identifier, ou au moins juger les homologies de tels parasites, plus particulierement de ceux possedant le même habitat, comme les spirochetes dont il s'agit ici

C'est pour cela que je me suis plus particulierement adonne a l étude des méthodes morphologiques qui pussent rendre service aux microscopistes Et ayant employé tous les moyens pour une etude cytologique détaillee et ayant remarque que les dimensions maxima et minima ne disent en general rien, car elles constituent dans la biologie des Spirochetes des exceptions, meme sans prendre compte des formes geantes si frequentes chez de tels parasites et que ce qui nous donne une idee plus juste de tels protistes ce sont exactement les dimensions de la plupart je suis par hasard arrivé a faire une constatation interessante(2) en etudiant les spirochetes des arcades dentaires humaines

(*a*) qu'il y avait des spirochetes larges a spires laches dont la longueur etait a peu pres deux fois plus grande que le nombre de leurs spires, (*b*) qu'il y en avait d autres, assez minces et a spires serrees dont la longueur etait a peu pres égale au nombre de leurs spires

La premiere espece avec ces caracteres toujours constants etait le *Sp buccalis* Cohn, la seconde le *Sp dentium* de Kock

J'ai alors voulu voir si cette rélation entre la longueur et le nombre des spires—relation prise sur les moyennes d au moins cent spirochetes d'à peu pres la meme largeur dessines à chambre claire—pourrait etre utilisee avec profit pour la caractérisation des especes *Cette relation qui a eté appelée indice d identification morphologique*(3) a donne issue a de nombreux travaux la rendant de plus en plus compliquée employant même à titre de la simplifier, des formules et des calculs algébriques !(4, 5) Et on a pu conclure que les resultats obtenus par cet *indice* n'étaient pas constants

Est modus in rebus ! Loin de moi affirmer que je puisse identifier par ce procedé les spirochetes de l intestion d un Termite vis a vis de ceux des arcades dentaires humaines ! Encore une autre remarque il y a souvent des spirochetes qui au cours

de leur trajet ont sur le meme individu des courbes irregulieres, les unes à grand rayon, les autres à petit rayon Mais en nous fixant sur les spirochetes qui nous occupent et qui ont des spires si regulieres si j etale sur une lame une goutte de pyorrhee alveolaire j'y fait quatre groupes suivant leurs largeurs, et mésure chaque groupe en tenant compte de leurs longueurs du nombre de leurs spires et de leur *index relation*, je peux m'assurer que les groupes sont suffisament caracterises beaucoup plus rigoureusement qu auparavant et que je peux maintenant les comparer avec les fins spirochetes de l intestin du meme sujet dans la certitude autant qu'il peut y avoir des certitudes en biologie[1] —que les differences que j obtiens m habilitent a juger que l espece *Euryggyrata* est différente des especes buccales

Davantage si dans l intestin humain à cote du fin *Euryggyrata* je trouve une large espece coprophytique dont les dimensions correspondent a l une des especes buccales du meme individu je me crois autorise non seulement à non considérer cette large espece comme un polymorphisme de l *Euryggyrata* mais comme une forme buccale ayant passé dans l intestin et conservé dans cet organe inalterés ses caracteres

J insiste donc sur l utilite des methodes morphologiques pour la caractérisation de tels spirochetes en ne leur demandant neanmoins plus qu elles peuvent donner dans ces etudes et faute de cultures pures de souches pures sur lesquelles je ne suis pas pour le moment en mesure de me prononcer

Methodes employees

Ceci posé je passerai a decrire les methodes employees dans cette caracterisation

(1) étude cytologique detaillée du parasite sur frottis colores par differentes methodes sans oublier les fixations humides et les colorations par l'hematoxyline a fer afin de bien saisir la structure du parasite Souvent des spirochetes ayant des caracteres tout a fait semblables different par l existence ou non existence soit d une membrane rudimentaire soit d un flagelle terminal ce qui nous aide a classifier le genre dans lequel le parasite doit etre inclus

(2) la notation des pourcentages des longueurs et du nombre des spires pris sur au moins cent spirochetes déssinés a chambre claire et mésurés suivant leurs tours de spires On resume enfin ces eléments de façon a avoir la maxima la minima et les oscillations de la plupart

(3) la largeur prise sur des préparations colorees en indiquant toujours la technique employée puisque cet element varie selon les solutions employées ainsi, les solutions phéniquees donnent une largeur bien supérieure a celle des colorants dérives du Romanowsky

Cet élement represente le premier facteur de separation apres que l étude cytologique nous habilite à la classification du genre lorsqu on trouve dans la préparation plusieurs especes de spirochetes

(4) le rapport entre la moyenne des longueurs et la moyenne du nombre des spires (notre *indice d identification morphologique*) les moyennes etant prises sur tous les spirochetes dessines

SPIROCHETES DES ARCADES ALVEOLO DENTAIRES DE L'HOMME

Aux Indes Portugaise ces spirochetes se rencontrent à peu pres dans toutes l bouches avec une abondance extraordinaire, néanmoins, dans les cas de pyorrh alveolaire Cette maladie est tres fréquente dans notre Inde comme on peut voir des chiffres suivants

AGES			GROUPES SOCIAUX		SEXES	
3 12 ans	12 21 ans	Au delà de 21 ans	Chretiens	Hindous et Maures	Hommes	Femmes
Pour cent 20	Pour cent 50	Pour cent 80	Pour cent 85	Pour cent 80	Pour cent 80	Pour cent 30

Ces spirochetes ont leur habitat dans les arcades dentaires, mais se trouver neanmoins dans d autres regions de la bouche en nombre infiniment inférieur, comm on peut voir des chiffres suivants, pris a 100 champs microscopiques

	Cas 1	Cas 2	Cas 3
Arcade dentaire	21	16	31
Salive	6	6	5
Voute palatine	0 1	0 2	0 1
Amygdales	0 1	0 19	0 0[illegible]
Rhynopharynx	0 02	0 05	0 04

Les chiffres concernant ces spirochetes sont resumés dans le tableau a suivr (chiffres pris a Angola)

Type	Caractères résumés	Largeur	LONGUEUR			NOMBRE DES SPIRES			Index
			Min	Max	Majorité	Min	Max	Majorité	
I	Espece large à spires laches	0 5 1	5	18	Pour cent 72 10—15	3	14	Pour cent 85 4—7	2 21
II	Mince à spires serrées	0 2 0 4	5	16	Pour cent 89 6—12	4	15	Pour cent 91 6—11	1 4

Type	Caractères résumés	Largeur	Longueur			Nombre des Spires			Index
			Min	Max	Majorité	Min	Max	Majorité	
III	Large à spires serrées	0 4-0 7	5	24	Pour cent 72 / 8—15	4	16	Pour cent 83 / 5—11	1 49
IV	Mince à spires larges	0 2-0 4	4	26	Pour cent 72 / 8—15	2	11	Pour cent 86 / 3—7	2 3

Le type I est le type *Buccalis*, le type II le *Dentium*, le type III *Intermedium*, le type IV semble une *Var Buccalis* plus mince

A' l'Inde Portugaise, les especes *Buccalis* et *Dentium* sont tres abondantes L'espece *Intermedium* est plus rare et la *Var Buccalis* n'a pas ete rencontrée dans nos recherches Les chiffres qui leur concernent subissent des oscillations qui n'infirment neanmoins les caracteres résumes que nous avons groupés dans la colonne respective du tableau supra, comme on peut voir ci dessous

Type	Caractères résumés	Largeur	Longueur			Nombre des Spires			Index
			Min	Max	Majorité	Min	Max	Majorité	
I	Large à spires laches	0 5-1	5	19 25	Pour cent 75—89 / 8—16	2	11	Pour cent 80—91 / 4—7	2 1 a 2 78
II	Mince à spires serres	0 25-0 40	5	18	Pour cent 75 / 6—11	[illegible]	20	Pour cent 82—90 / 6—13	0 87 a 1 4
III	Large a spires serrés	0 4-0 8	[illegible]	18 27	Pour cent 75—85 / 7—15	3	19	Pour cent 85 / 6—12	1 5 à 1 7

Spirochetes des arcades dentaires des animaux

Plusieurs especes animales ont dans leurs arcades dentaires une faune spirochétienne absolument identique a celle des arcades humaines, sans qu'ils présentent le moindre signe d'altération de santé

Les rats les souris les chévres les moutons ne nous ont pas montre des spirochétes

Les éléments concernant les animaux qui ont montré de résultats positifs sont groupés dans le tableau à suivre (Tableau I)

Tableau I.

Spirochetes des arcades dentaires des animaux

Espèce animale	Animaux examinés	Résultat positif	Pourcentage de l'infestation d'après l'examen de 100 champs micr (par champ)	Caracteres résumés des spirochetes	Largeur	Longueur			Nombre des Spires			Index	Ressemblance avec les especès humaines
						Min	Max	Majorité	Min	Max	Majorité		
Cheval ..	10	8	Type I, 0 38 à 6 2	Gros à spires larges	0 6-0 8	3	20	Pour cent 82, 5–10	2	9	Pour cent 85, 3–6	1 7	Buccalis
			„ II, 0 11 à 2 4	Fin à spires laches	0 25	4	13	Pour cent 92, 5–10	2	11	Pour cent 96, 3–7	1 3	Var Buccalis
			„ III, 0 06 à 2 1	Gros à spires serrées	0 3-0 5	3	16	Pour cent 80, 5–10	3	15	Pour cent 87, 4–10	0 99	Intermedium.
			„ IV, 0 06 à 0 6	Fin à spires serrées	0 25	3	14	Pour cent 88, 6–11	5	14	Pour cent 80, 6–11	0 90	Dentium
Porc .	3	2	Type I, 0 36 à 0 63	Gros à spires larges.	0 5-0 7	5	19	Pour cent 77, 7–12	3	14	Pour cent 83, 4–9	1 5	Buccalis

		„ II 0 à 0 46	Gros à spires serrées	0 4–0 6	6	18	Pour cent 74 / 8–12	[illegible]	19	Pour cent 93 / 9–16	0 9	Intermedium
		„ III, 0 à 0 6	Fin à spires serrées	0 25 0 40	5	18	Pour cent 70 / 8–11	3	15	Pour cent 93 / 5–10	1 18	Dentium
Bœuf	4	2 Type I, 5 7 à 6	Gros à spires larges	0 7–0 8	6	15	Pour cent 80 / [illegible]–13	3	11	Pour cent 81 / 5–8	1 4	Buccalis
		„ II, 3 9 à 4 2	Fin à spires serrées	0 25–0 35	5	15	Pour cent 77 / 7–8	6	18	Pour cent 76 / 8–13	0 8	Dentium
Chien	2	2 Type I, 1 9 à 2 4	Gros à spires larges	0 6–0 8	5	19	Pour cent 70 / 7–11	3	12	Pour cent 92 / 3–9	1 5	Buccalis
		„ II, 0 56 à 0 66	Gros à spires serrées	0 4–0 6	5	16	Pour cent 79 / 7–12	7	20	Pour cent 82 / 8–15	0 80	Intermediaires
		„ III, 0 56 à 0 66	Fin à spires lâches	0 25–0 30	4	18	Pour cent 67 / 8–12	3	14	Pour cent 78 / 4–8	1 1	Intermediaires
		„ IV, 0 55 à 1 1	Fin à spires serrées	0 25	5	16	Pour cent 76 / 7–11	6	20	Pour cent 78 / 10–15	0 7	Dentium

SPIROCHÈTES DE L'INTESTIN DE L'HOMME

Plusieurs spirochètes peuvent parasiter l'intestin humain

(*a*) les uns trouvés fortuitement comme le *Sp macfiei* mihi 1917 un gros spiro-
chète ressemblant à des organismes similaires que l'on rencontre dans l'intestin de
crapauds le *S Couceiri* mihi 1920 vibrio spirochète trouvé une fois dans les selle
d'une dame avec dysenterie chronique à amibes et flagellés ce n'est pas sur ceux c
que nous voulons appeler l'attention

(*b*) d'autres qui sont nettement des *spirochetes buccaux* qui passent dan
l'intestin et y gardent inaltérés leurs caractères Ce fait doit nous mettre en gard
contre la prétendue validité et autonomie des espèces coprophytiques si on n'a pa
eu le soin de les comparer avec leurs congénères buccales

Les caractères morphologiques et les dimensions des spirochètes buccau
passés dans la cavité intestinale peuvent servir pour une bonne différentiation d
tels organismes vis à vis du spirochète intestinal par excellence ou le *S eurygyrat*
Werner *emend* Fantham

Le tableau à suivre est assez élucidatif et concerne un enfant dont les selle
nous ont montré à côté de rares *Eurygyrata* une large espèce coprophytique qui s
montrant structuralement identique à l'espèce *Buccalis* nous donna des chiffre
très intéressants en comparaison avec les espèces buccales du même individu A
remarquer que de tels cas sont très rares dans ma statistique de plus de 500 selle
je ne compte que cinq cas à peine !

	Espèce intestinal	Buccalis	Dentium	Intermedium	
Min	[illegible]	6	5	5	Longueur
Max	19	18	18	18	
Maj	Pour cent 88 / 8–16	Pour cent 88 / 6–12	Pour cent 76 / 6–11	Pour cent 93 / 7–16	
Min	3	2	5	5	Nombre de spires
Max	11	6	0	1[illegible]	
Maj	Pour cent 96 / 4–9	Pour cent 93 / 3–6	Pour cent 95 / 6–11	Pour cent 8[illegible] / 6–12	
		3	1 0	1 17	I[illegible]les

Cette espèce intestinale s'approche du *Buccalis* et si on ne trouve pas une par
faite exactitude c'est d'abord parce que ces méthodes ne sont pas mathématiques

et ensuite parce que quelques autres spirochetes buccaux des types *Intermedium* et *Dentium* passant dans l intestin sont aussi comptés dans la caractérisation de cette soidisant espece intestinale et faussent ainsi les résultats d une telle caractérisation(6)

Voici pourquoi les figures données par Mr le Dantec sur les agents de la Dysenterie spirillaire nous semblent appartenir à au moins deux types les uns de 6 à 14 microns au type *Eurygyrata* et d autres de 30 a 40 microns a un type different une longue espece coprophytique peut etre meme d origine buccale(7)

On trouvera donc justifiable le doute qui s empare de mon esprit pour accepter sans objections la théorie qui fait l espece *Eurygyrata* une variete intestinale des especes buccales !

(c) L espece intestinale ubiquiste cosmopolite au meme titre que le *Bacterium coli* est le *S eurygyrata* Son abondance dans l intestin humain subit d s oscillations qui ne sont souvent en rapport avec les états entéritiques Ainsi les chiffres a suivre sont élucidatifs

Individus normaux	
Examinés	51
Sans sp	2
Avec sp	49
Pourcentage	96 2

Individus entéritiques

Enterite vermineuse 5 cas tous positifs 100 pour cent
Entérite tuberculeuse 2 cas dont 1 positif 50 pour cent
Dysenterie amébienne 6 cas dont 1 negatif 83 pour cent
Ankylostomose et flagelose 1 cas positif
Diarrhée probablement balantidienne 1 cas positif
Total d individus entéritiques 15 dont 3 negatifs
Pourcentage 80

Il faut remarquer que dans 6 selles cholériques examinées a part j ai trouvé une abondante infestation par l *Eurygyrata*

L indice meme de cette infestation varie selon les individus comme on peut le voir du tableau suivant —

Nombre des spirochètes par chaque champ microscopique	Individus normaux	Individus enteritiques
1 à 5	19 cas 38 pour cent	5 cas 50 pour cent
5 à 10	8 16	1 10
10 à 15	6 12	1 10
15 à 20	6 12	1 10
20 à 50	7 14	2 20
au dessus de 50 ou incomptables	3 6 »	

Des spirochetes absolument identiques sont trouvés dans les selles des animaux qui montrent aussi quelquefois bien que rarement d autres especes auxquelles s appliquent toutes les remarques que je viens de faire a propos des especes intestinales en général Encore une note non seulement l indice de cette infestation varie suivant l individu mais encore une espece animale montrant l *Eurygyrata* dans un pays peut etre indemne dans un autre(8 9 10)

Le tableau à suivre resume tous les éléments concernant l espece *Eurygyrata* de l intestin de l homme et des animaux domestiques (Tableau II)

TABLEAU II

Caractères morphologiques des Spirochetes du type Eurygyrata *de l'intestin de l'homme et des animaux*

Espèce.	Provenance.	Largeur	Longueur			Nombre des Spires			Index.	Observations
			Min	Max	Majorité	Min	Max.	Majorité		
Homme ..	Inde Portugaise	0 2 (au Romanosky 0 25 à 0 30 en solutions pheniquées	3	11	Pour cent $\frac{83-91}{4-9}$	2	13	Pour cent $\frac{77-83}{3-8}$	1 13 à 1 19	..
„ ..	Angola	0 20 à 0 25	3	11	Pour cent $\frac{77-82}{4-7}$	2	11	Pour cent $\frac{86}{3-7}$	0 9 à 1 2	On trouve aussi dans l'intestins des indigènes d'Angola des types plus longs appartenant à une espèce animale peut être chèvre.
Cheval ..	I. P	0 25-0 35	4	12	Pour cent $\frac{78}{7-10}$	2	9	Pour cent $\frac{93}{4-8}$	1 4	....
„ ..	Angola	0 20-0 40	4	11	Pour cent $\frac{92}{4-8}$	3	7	Pour cent $\frac{92}{3-6}$	1 4	...
Mouton ..	I. P.	0 25-0 40	2	9	Pour cent $\frac{90}{3-6}$	2	9	Pour cent $\frac{95}{3-7}$	0 9	....
„ ..	Angola	0 25-0 40	3	14	Pour cent $\frac{89}{5-10}$	2	8	Pour cent $\frac{92}{3-6}$	1 24	.

Porc	I P	0 25-0 30	3	9	Pour cent 97 / 4–8	2	9	Pour cent 88 / 4–7	1 04	
"	Angola	?	3	10	Pour cent 85 / 5–8	2	7	Pour cent 86 / 3–5	1 6	
Souris blanche	I P Type I	0 15-0 20	2	9	Pour cent 93 / 3–7	2	10	Pour cent 91 / 3–8	0 8	
"	" " II	0 25-0 30	3	13	Pour cent 88 / 4–9	2	12	Pour cent 95 / 3–7	1 3	S sp ?
Chèvre	I P	0 25	2	9	Pour cent 86 / 4–7	3	12	Pour cent 89 / 4–8	0 9	
"	Angola	0 25-0 40	4	15	Pour cent 80 / 5–10	3	14	Pour cent 79 / 5–[illegible]	0 16	
Bœuf	I P	0 4	4	11	Pour cent 94 / 4–8	2	10	Pour cent 86 / 3–6	1 1	
"	Angola	0 3-0 7	4	15	Pour cent 74 / 5–10	3	10	Pour cent 90 / 4–8	1 4	
Chien	Angola	?	4	13	Pour cent 94 / 5–10	3	8	Pour cent 86 / 3–6	1 3	Nous n'avons pas trouvé des sp de ce type dans l'intestin des chiens à I P

TABLEAU II—*fin*

Espèce	Provenance	Largeur	Longueur			Nombre des Spires			Index	Observations
			Min	Max	Majorité	Min	Max	Majorité		
Rat (M Rattus)	I P	0 20	2	5	Pour cent 98 2–4	2	6	Pour cent 93 2–4	0 8	
Lapin .	Angola	0 20–0 40	3	10	Pour cent 93 5–8	2	7	Pour cent 98 ,–6	1 3	Pas trouvé dans l'Inde Portugaise
Cobaye	Angola	0 2–0 4	3	10	Pour cent 88 4–8	2	8	Pour cent 96 1–6	1 58	..
Mule .	Angola	0 2–0 4	4	12	Pour cent 93 6–11	3	8	Pour cent 87 3–6	1 7	
Chat ..	Angola	0 25–0 50	3	10	Pour cent 88 5–8	2	7	Pour cent 96 3–6	1 4	

CONCLUSION

I I es arcades dentaires de plusieurs mammiferes hebergent une faune spirochétique entierement semblable a celle des arcades dentaires humaines

II I intestin de plusieurs mammiferes domestiques heberge une faune spirochétique tres ressemblante au *S eurygyrata* humain

III Les methodes morphologiques peuvent servir pour caractériser ces especes ou au moins pour montrer leurs homologies dans l échelle animale

IV Il faut tenir compte de ce commensalisme lorsqu il s agit de creer d entites morbides *soidisant* spirochetiennes

INDEX BIBLIOGRAPHIQUE

(1) SANARELLI C (1927) Les sp rochetes cutanes *Ann de l Ins Pasteur*

(2) FRO LANO DE MELI ET LAKSHMANA IADD IA (1923) Sp rochètes les cav és b ccales h n a nes à l Inde Portuga se et leurs r lat ns avec l pyorrh e alveo la re *A Mel Moderna Porto*

(3) I RO LANO DE MELLO ET MARIO D AN RA E (19) Sp ro hètose broncho pulmon re au Nor l le Port al *B ll Soc Path Exot*

(4) A TO N (19 4) Men u at ons des co rb res des sp rocl tes *C mpt Rend Soc B ol*

(5) DELAMARE G (19 4 1926) Au sujet de l ndex d dent f cat on morpholo q e les sp rochetes bron h ques *Ib d*

(6) BRUN PERE RA ME Q ITA (19) F p ro l etoses ntest na s l umanas *Arqu Indo Port de Med e Hist N t*

(7) LE DANTEC A P c s le Patholo e Exot q e 4ème é l t

(8) I RO LAN DE MELLO (1 4) Pr m ere cont but on à l étude d s Sp rocl t l s d Angola *C mpt rend du I r n Congr de M l Trop da Af a occ dent R Mel d Angola*

(9) *I lem* (19 5) Sp rochetose broncl o p lmona re *Arq Indo Po t de Mel e H st Nat*

(10) *I l m* ET MESQ TA B I (19 4) p rochéto es ntest nales l n a nes à l In le l rt aise *B l Soc P th Exot*

THE CRYPTOCOCCUS

BY

K BANERJEE M B, D T M,

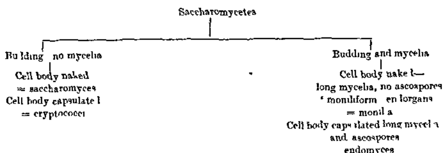
Assistant Professor of Pathology Calcutta School of Tropical Medicine and Hygiene

RIDAY, EC 9TH TO 4 P M

MANY yeast like parasites have been isolated by various workers both in m logy and bacteriology from pathological exudates and the names that have l given to them have been based mostly on cultural and not on morpholog characteristics Workers in the tropics have come across these yeast like bo *perhaps more often than their confreres in temperate climates, as some of t* organisms can be isolated from the throat, tonsils, sputum stool, urine skin t and nose etc under suitable conditions of temperature and humidity frequency of their occurrence and their wide distribution in nature have led n observers to look upon them as non pathogenic contaminations This is n probably the reason why some of the names, both generic and specific, have l so confusing A pure culture of one of the species is sometimes isolated by a wo who makes a few notes on its cultural behaviour and gives it a name before life cycle has been completely worked out The term *monilia* seems to fit mos the organisms of this class and any thick creamy growth especially on car hydrate media which shows a certain amount of budding in a fresh smear has b usually classed under this genus Some observers choose to call all these ty of organisms *saccharomyces* while others often confuse the genera *blastomy cryptococcus* and *saccharomyces* as one This state of affairs certainly does *improve when the monilia are also included in this list In point of fact* entire subject is in a state of chaos and the average worker in a laboratory l the greatest difficulty in finding the correct generic or specific name for any yea like culture that he may cultivate from a lesion

The *cryptococcus* belongs to this class of saccharomycetes which has confus many a careful and clever worker The name cryptococcus (from the Greek wo κρυπτος meaning hidden) was given by its discoverer Kutzing (1833) on accou of the life history of the organism being practically unknown It has be described as an unicellular capsulated circular or ovoid organism measuring abo 12 to 16 μ along its longest diameter, the cell body containing some refract granule presumably plant volutin There is a larger or smaller bud attached to t parent cell at its periphery Its recognition as a pathogenic parasite in the trop

is due to Castellani who described a peculiar cutaneous lesion, cryptococcal dermatitis, caused by cryptococcus the *Cryptococcus hominis* The elaborate classification of the different species, though very confusing for the beginner in mycology, gives at least order in a region which is more or less unknown In this short paper the writer has kept to the fundamental classification as mapped out by Castellani



In our laboratory the first case of cryptococcal dermatitis which came under observation was a diabetic patient who was asked to consult Col H W Acton for a 'very aggravated persistently pustular prickly heat' affecting the skin of his abdomen only Needless to say, the term 'prickly heat' was used in a loose sense in this case as the patient came under observation when the cold weather was setting in

Nature of the lesion —The eruption was quite discreet and each pustular vesicle was surrounded by a circumscribed patch of pink areole the pustules themselves being pearly white and opaque There was no actual pain although the patient complained of a good deal of discomfort and deep seated tenderness This pink areole is seen only in fair European skins The skin over the vesicles was quite thin and soft, so that it could be easily lifted up with the platinum needle liberating a drop of thick creamy white pus A thin smear from the pus examined in the fresh state under the microscope showed numerous double contoured spherical or slightly ovoid cryptococcus like' organisms with a small bud attached to the outer wall of the parent cell No mycelia could be found after a very careful search even in stained specimens and secondary infective bacteria like *streptococci* and *staphylococci* were entirely absent Cultivated on glucose and saccharose agar, it gave a thick opaque creamy white growth which on examination showed the typical cryptococcal appearance with budding The capsule was absent in a six days old culture We saw similar lesions on four other occasions in patients three of which were diabetics and the fourth was suffering from sprue The cryptococcus was isolated in pure culture in every case

So far both the skin lesions and the organisms isolated from them, resemble the description of cryptococcal dermatitis and cryptococci given by Castellani in his book on 'Tropical Medicine' and, on the basis of this description all our cases were

diagnosed as cryptococcal dermatitis Clinically our cases also bear a very close relationship to those reported by Smith in the *Transactions of the Royal Society of Tropical Medicine* diagnosed by him as 'prickly heat' To the residents in the tropics prickly heat is not an uncommon skin affection and those of us in Bengal have had the opportunity of studying hundreds of cases for at least five months every year Col Acton has shown that 'prickly heat' is a staphylococcal infection of the sudden sweat glands secondary to seborrhœic dermatitis which is caused by an entirely different family—the *Malassezia*—commonly called the 'bottle bacilli' but even the worst cases *of prickly heat never approach the clinical* appearances described by Smith (1927) One may ask 'why this particular kind of prickly heat as described by Smith is so uncommon amongst the inhabitants of Bengal during the summer and early rainy season In all probability Smith's cases belong to the group at present under notice and are not prickly heat In order to study the so called cryptococcus isolated agar slope cultures were examined every fourth day No striking morphological changes appeared in the organisms *The older cultures had a shrunken appearance and sometimes a kind of* vacuole appeared in the centre of the large coccus like body For the first five to six days the organisms were more or less spherical with a fairly large circular bud attached to the outer wall and as the medium showed signs of drying up the individual organisms became more and more oval in shape and showed two or three small buds attached to the parent cell

Cultural characteristics—The organism grows very well under ærobic conditions on all ordinary media but on media containing carbohydrates like saccharose agar glucose agar or Sabouraud's media the growth is more vigorous On blood agar there is a distinct zone of white hæmolysis On McConkey's media the bile salts exert an inhibitory action and the colonies appear as small opaque lactose fermenters In ordinary broth the growth is not very abundant

Influence of high salt content of the media—This organism was cultivated on glucose agar containing 0 5 1 0 1 5 2 0 and 2 5 per cent of sodium chloride There was a certain amount of inhibition in media containing 2 and 2 5 per cent of the salt

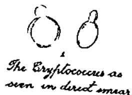
Influence of pH—Glucose agars of pH 5 0 5 5 6 0 6 5 7 0 7 5 8 0 8 5 9 0 9 5 and 10 0 respectively were sewn with the culture In 48 hours the growth was most abundant in pH 6 6 5 and 7 Marked inhibition was noticed on the strongly alkaline side The optimum growth is between pH 6 and 7

Influence of staphylococci—An ordinary agar slope was first planted with *staphylococcus aureus* and after 24 hours the culture was carefully scraped off the slope A large loopful of the organism was then sewn on the media and incubated for 48 hours The growth was very markedly inhibited

Media containing urea have no inhibitory effect at all

Sugar reactions—The organism is a lactose fermenter producing acid and gas Glucose, maltose and mannite are also fermented by it with the production of acid

PLATE VI



1
The Cryptococcus as seen in direct smear



2
Primary Culture



3
Appearance in old agar culture

4
Different stages in the morphological change

1 2 3 4 5 6 7 8

and gas Dulcite salicium and saccharose are not fermented and litmus milk remains alkaline after 48 hours

Anærobic cultivation —Partial anærobiasis in culture tubes has very little effect on the growth Cultivated in Mackintosh Field s strict anærobic apparatus the growth is not hindered to any great extent

Effect of heat and of chemicals —Heated to 56°C , the culture is killed in ten minutes Half per cent absolute phenol takes about half an hour to kill this organism

Morphological changes —The earliest signs of morphological change were noticed when a hanging drop preparation was put up with a 2 per cent saccharose saline and examined after about six hours Some of the smaller buds seemed to be replaced by a long tapering rod , no vacuole appeared in the centre of the parent cell and the rod itself showed a few highly refractile granules inside A flask containing about 100 c cs of Raulin s medium was inoculated from an old culture tube and the growth examined by the hanging drop method from day to day At first there was a very small flaky light yellowish deposit at the bottom and as the growth proceeded the appearance was like that of moss leaving the media generally clear The earliest transitional stage was noticed on the third day when a large bud from the parent cell seemed to lengthen itself out like a mycelial rod and the refractile granules were more noticeable in both the mother cell and the mycelium On the fourth day, distinct septate mycelia appeared in the flask and multiplication by budding had almost entirely ceased [illegible]

old agar [illegible]

hours if [illegible]

containing the minimum amount of nutrition (like Raulin's) mycelial elements containing volutin granules begin to appear from the third day onwards On the fourth day the mycelia become septate and lateral germinules make their appearance at the nodes In ten days' time the growth is a mass of long interlacing septate mycelia with two to six visible germinules at the nodes Some of the mycelial rods show a series of three or four buds at the tip and thus closely resemble a monilia while others show an ovoid or spherical endorgan rich in volutin These rounded endorgans break off from the mycelia and multiply first by means of two or three large buds which again form the long septate mycelial elements Multiplication thus goes on by mycelial formation so long as there is enough of nutrition available in the media

These inoculated flasks were examined again after two months when the nutrition of the media was very nearly exhausted Multiplication by septate mycelia had nearly ceased and there appeared in the place of the spherical endorgan a fairly large ascospore with a fairly thick double contoured perithecium containing four asci The ascospore breaks off the perithecium ruptures and the asci are liberated When plenty of nutrition is again available most likely on a human host these asci take up the cycle of the second generation

The writer had now to find a suitable name for this organism The lesion from which it was isolated was exactly like a cryptococcal dermatitis, and the morphological characters in cultures on solid media conformed in every respect with the book description of a typical cryptococcus When put into fluid media containing carbohydrates *in suitable percentage, however, it entirely* changes its morphology and develops septate mycelia, germinules and moniliform endorgans which a cryptococcus according to the classification never does We have confirmed this by cultivating a specimen of *Cryptococcus hominis* sent to us from London *in* Raulin's media Then again the formation of an ascospore with four asci definitely excludes it from the group monilia

Most valuable help was obtained from Col Acton's paper on 'Endomyces Tropicalis, the causative organism of tropical sore throat' published in the *Indian Journal of Medical Research* in 1918 19 The behaviour and metamorphosis of our so called cryptococcus closely follows that of the *Endomyces tropicalis* and the similarity of the two is very close except in points of minute difference in the size and shape of the mycelia, germinules and ascospores Based on these observations, the organism is an endomyces and not a cryptococcus in the true sense

All our attempts to reproduce the disease by inoculations with the culture into both human and animal hosts have given negative results Subcutaneous injection of 2 c cs of a thick emulsion in 2 per cent saccharose saline into the abdomen of guinea pigs and rabbits produced a dry looking punched out ulcer at the site of inoculation Smears taken from the edge as well as the centre did *not show any cryptococcal forms and cultures from different parts of the sore* gave negative results as well On human subjects the skin at the flexure of the elbows was scarified with a sharp knife and a drop of thick emulsion of the organism in 2 per cent saccharose saline was allowed to dry on this spot Excepting for a mild erythema on account of the scratching, there was no effect of the inoculation on the skin

Conclusions

(1) Lesions very similar to cryptococcal dermatitis can be produced by other members of the saccharomycetes group

(2) The endomyces usually multiplies by budding It is not a monilia although a ten days' growth in Raulin's media shows moniliform endorgans

(3) According to the characteristics of the genus it shows septate mycelia nodal germinules and ascospores with four asci in Raulin's media and has therefore been termed 'endomyces'

The writer, who is not a mycologist, found great difficulty in connection with the subject matter of this paper Col H W Acton kindly advised as to the lines along which the investigation should be carried on and the author takes the opportunity to express his grateful indebtedness and thanks to him

DISCUSSION

Col I Froilano de Mello (Portuguese India) Congratulated the author on his interesting paper and thought that the fungus perhaps could not be classified among the cryptococci, as the author found asci He recommended that the study of the fungus be continued so that a complete knowledge of its mycological evolution be obtained when its generic classification would be an easy one

Dr K Bannerjee (Bengal) replied The heading of the paper was based on the findings in the smear from cases of what was apparently cryptococcal dermatitis which conformed to the description given by Castellani The classification is admittedly not perfect and is not based on biological characteristics Col de Mello s lucid and learned classification based on cultural characters opens out possibilities for better and more accurate diagnosis and identification of the yeast like bodies that are found in many normal and morbid exudates His help will certainly be greatly valued by the author

NOTE ON THE PREPARATION OF MUTTON BROTH WITH PAPAIN

BY

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DURING the early part of our work on bacteriophage at the Pasteur Institute Rangoon we used Martin's broth exclusively this medium being recommended by Dr d'Herelle as the most suitable for the purpose

When however we commenced the preparation of bacteriophage on a comparatively large scale for testing its therapeutic value in the treatment of bacillary dysentery it became evident that a substitute for Martin's broth would have to be devised a substitute that could be administered to all communities easily prepared and of low cost

Lieut Col J Morison suggested that papain the dried juice of the papaya fruit might prove to be an efficient substitute for pigs stomachs in the preparation of Martin's broth A sample of papain was procured from Ceylon and the following tests carried out

Twenty five grammes of finely minced beef and 100 c cs of water were placed in each of a number of 200 c c flasks A quantity of papain was ground up in a mortar and from this gradually increasing quantities ranging from 0.5 per cent to 15 per cent of the weight of mince taken were added to each flask The flasks were then placed in a water bath at 60°C for six hours and at the end of that time the amount of digestion noted A second series of flasks were put up in the same manner except that in this case the contents of the flasks were acidulated with dilute hydrochloric acid At the end of six hours broths were prepared from each flask and tested for its power to grow dysentery bacilli and also for the degree of lysis that took place when suspensions of dysentery bacilli were acted on by bacteriophage

It has been found by repeated experiments that the best broth is obtained when about 6 grammes of papain are added to 100 grammes of mince without the addition of acid the papain itself being decidedly acid to litmus paper More rapid and complete digestion takes place when the temperature of the water bath instead of being maintained at 60°C is gradually raised to 80° C after two or more hours at the lower temperature

The next step was to substitute mutton mince for beef The experiments were repeated and found to correspond in every way to those done with beef mince The following is our routine method for preparing mutton broth and we have been doing this for several months

STOCK A

1 Rub up 300 grammes of mutton mince freed from fat, in a mortar with 20 grammes of powdered papain

2 Stir in gradually 200 c cs of distilled water and transfer the whole to a large flask Add 1 1 litre of distilled water

3 Place the flask in a water bath at 50° C for two hours and during the next two hours gradually raise the temperature to 80°C This latter temperature should be maintained for two hours i e till six hours in all have been completed The flasks should be well shaken every hour

4 Raise the temperature to boiling point to stop further action of the papain and cool

5 Strain through a thick wet cloth, make distinctly alkaline to litmus by adding a sufficient quantity of normal caustic soda and steam in the steamer for 30 minutes Cool

6 Filter through Kieselgurh deposited on filter paper

STOCK B

1 Add 500 grammes of mutton mince to a litre of distilled water and steam for one hour

2 Strain through a wet cloth make distinctly alkaline to litmus, steam for 30 minutes and filter through filter paper

When required for use, mix equal parts of A and B Steam for 30 minutes, cool and filter Adjust the hydrogen ion concentration to 7 8, tube or place in flasks and autoclave

The broth should be perfectly clear and of a light golden colour

The cost of preparing a litre of this broth in Rangoon is roughly Rs 1 6 against Rs 3 2 for the same amount of Martin's broth

Individual samples of papain vary somewhat in their digestive powers Each sample should be tested on receipt from the makers The minimum amount giving complete digestion in 6 hours should be used Excess of papain apart from being wasteful gives rise to a heavy precipitate on the addition of caustic soda when making the broth alkaline to litmus This necessitates more frequent filtrations It also tends to darken the colour of the broth giving it a somewhat greenish tinge

CONCLUSIONS

In our hands this mutton broth has given as good results as Martin s broth, a good bacillary growth is obtained and lysis takes place rapidly and completely on the addition of bacteriophage

It is not open to the obvious objections to Martin's broth when used for internal administration in the East

The cost of preparation is considerably below that of Martin's broth

It can be prepared in half the time

I am indebted to Lieut Col J Morison, I M S, Director of the Pasteur Institute, for his help and many valuable suggestions

/

ON THE ANÆROBIC BACTERIAL FLORA OF CERTAIN CASES OF CELLULITIS AND GANGRENE

BY

A C UKIL

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IN the absence of any inquiry as to the incidence of anaerobic infections in wounds and inflammations in this country, it is difficult to say, from hospital returns what percentage of putrid and gangrenous lesions is due to association with anærobic bacteria For example, out of 251 cases of inflammations of the connective tissue ' admitted in two of the surgical hospitals of Calcutta in a single year, there were 36 cases of gangrene including seven cases of diabetic gangrene 120 cases of appendicitis or appendicular abscess 47 cases of deep seated cellulitis and 41 cases of other forms of cellulitis While it is difficult to say how many of these were due to anærobic bacteria, it is safe to state that in a large number of these cases anærobic bacteria were associated, as has already been demonstrated by workers in France and elsewhere A large number of them will be found not to have yielded either to sero therapy (against ærobic bacteria) or vaccino-therapy and to have ultimately proved fatal

That anærobic bacteria play a large part in the incidence of various infections in the tropics will be shown by the following data worked out by us —

Infections in the oral cavity —Fuso spirochætal association with or without spore bearing bacilli has been noticed in many cases of gingivitis, tonsillar ulcers and noma

Enteritis – Enteritis has been shown to be due to *B welchii* and *B sporogenes*(1) We have also found various spirochætes and spirilla in certain cases of diarrhœa, dysentery and frequently in the reaction stage in cholera We tried to isolate these spirochætes by various methods in Muhlen s and Noguchi's media and by filtration but our attempts have hitherto failed

Appendicitis —We have so far studied nine cases of appendicitis removed by surgical operation and have isolated *B welchii* in one case, *B œdematogene* in one case(2) and only ærobes in the rest (*B coli, streptococci enterococci*) We have been able to show the presence of the bacilli in the submucous layer in sections of the appendix A search for amœbic infection, either in its vegetative or cystic form, proved futile

Diabetic cellulitis and gangrene—Out of 12 cases of diabetic cellulitis the commonest organism which has been found is the staphylococcus next comes *B coli streptococcus*, and *B proteus* in order of frequency In cases with gangrene, we isolated *B sporogenes* only in one case in which it was associated with an overwhelming number of streptococci

Gangrene following injury—Out of ten cases studied, *B welchii* was isolated in seven cases *B vibrion septique* in two cases and a hitherto undescribed bacillus in one case(3) in association with streptococci, *B coli, Staphylococcus aureus, B proteus* and *B pyocyaneus* in order of frequency There was an aero anærobic association in all the cases, none of the cases showing multi anærobic or mono anærobic infections

Certain forms of cellulitis following abdominal operations and extravasation of urine—*B sporogenes* and some bacilli of mild pathogenicity(3) have been found in the non toxic but putrid forms

Lung infections—In lung gangrene, we have found fuso spirochætal association in two out of five cases observed In open' pulmonary tuberculosis, anaerobic streptococci and some species of anærobic gram positive cocci in clumps have been frequently found to be associated with various ærobic organisms These have been found to possess a moderate degree of pathogenicity on laboratory animals Fuso spirochætal association was found in very few cases

Besides the pathological conditions studied by us there are many other putrid or gangrenous inflammations of various channels of the body communicating with the exterior, body cavities or deeper tissues, which are caused by an association of anærobic organisms Thus, besides botulism and tetanus, we should look for these associations in the following conditions —

(1) Gangrene of a part following injury
(2) Affections of the alimentary canal, such as necrosis or gangrene of the mouth (gingivitis, pyorrhœa alveolaris, noma, angina), pharynx intestines, appendix, rectum or anus
(3) Affections of the respiratory passages, e g , gangrene and other fœtid conditions following pneumonia, influenza or tuberculosis
(4) Affections of the body cavities, e g , fœtid inflammation of pleural and peritoneal cavities
(5) Infection of the genito urinary passages (e g , puerperal sepsis, gangrene of the vulva, salpingitis, prostatitis and extravasation of urine)
(6) Putrid and gangrenous conditions in other parts of the body, such as diabetic carbuncle, gangrenous conditions of the skin, ear, mastoid abscess, cholecystitis, liver abscess, etc

In the absence of special knowledge, many of these conditions are treated in the tropics either by surgical measures alone or combined with or without anti ærobic sero therapy only But alas! most of these cases die owing to our ignorance of the real ætiological factors at play Anti toxic and anti bacterial sero therapy

has reduced the mortality of these conditions from 50 per cent to 15 per cent in France(4)

The more we study these cases the more we learn about them It is now known that many of the cases of appendicitis are due to anærobic bacterial associations *B welchii* (in one-third of the cases Weinberg) *B fallax* (in three out of [illegible] Duthie)(5) and *B œdematogene*(2) have been desc[illegible]

My object in speaking [illegible] in all tropical countries [illegible] morbidity and mortality in our medical [illegible] We do not yet know the normal bacteriological flora of the intestine of vegetarians, of whom there are many in India When a systematic study is begun in these countries we will perhaps understand the ætiology and pathology of many obscure conditions such as the condition of post puerperal diarrhœa alternating with constipation known as *Sootika* in India epidemic dropsy, some varieties of dyspepsia and enteritis, and the disease known as 'Black quarter' in cattle in India We must interpret the various inflammations and disease processes in terms of bacterial associations

REFERENCES

(1) Ukil, A C (1924) The role of anærobic bacteria in wounds and infections *Cal Med Jour* December

(2) *Idem* Un anærobie œdematogène de l'appendicite, *Compt Rend Soc de Biol* T LXXXVII p 1009

(3) *Idem* (1927) A preliminary note on anærobic infections in India *Cal Med Jour* August

(4) Weinberg, M (1927) Le serum anti gangreneux et son emploi en therapeutique, *Le Jour Med Franc* T XII February

(5) Duthie G M (1924) Presence du *B fallax* (Weinberg et Seguin) dans la flore de l'appendicite, *Compt Rend Soc de Biol* T XCI p 327 July

ACTINOMYCOSIS HOMINIS

BY

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The subject of my paper is actinomycosis hominis I prefer this name to many others as it conveys a better meaning and gives expression to the real character of the fungal organism

Mycosis is a condition of fungal infection and as this type of fungus shows a star shaped or radial arrangement of the hyphæ in a mycelium it is named actino myces and the disease is known as actinomycosis

This fungus is pathogenic to human beings and when introduced into the tissues it produces a lesion of the character of an infective granuloma often termed a mycetoma which literally means a fungus tumour During its infective process in the tissues it gives rise to variously shaped bodies called 'grains' which are found embedded in the tissues and often escape free in the discharges from the openings or sinuses in the diseased area These 'grains' look like fish roe bodies and are composed of very fine non septate mycelial filaments in which the walls are not clearly defined and never show any chlamydospores

The only infection with which it may be confounded is maduro mycosis This also gives rise to the formation of mycetoma with many sinuses discharging 'grains which are composed of large septate mycelial filaments possessing well defined walls and usually showing chlamydospores

Incidence —The incidence of human actinomycosis in India is regarded by many as of very rare occurrence as only a few cases are in the records but I hold the same view as Acland who has very truly remarked in writing about the geographical distribution of this infection that more cases are recognized where they are carefully looked for Since I published my first paper on this subject in the *Indian Medical Gazette* January 1918 in which I recorded my observations on three cases only I have come across nine more cases of this infection

Distribution —The distribution of the lesions in these cases is diverse in character Two of them were found in connection with the lung the pleura and the chest wall one affecting the female breast, two in the right foot and one in the right hand one affecting the right cheek and parotid region one affecting the left cervical and submaxillary glands and one was found in the deep muscles of the back on the right side near the lower dorsal spines

I had the opportunity to study some of these cases in detail and I believe it interesting to record certain facts which appear to me to be of a novel character.

I am unable to dwell, in detail, upon the history, progress of the disease and treatment of the individual cases and I can only relate those interesting features which will help us to make an early diagnosis of this infection

Out of the *nine* cases under my observation I would now mention only two in which the diagnosis was made early and the cases cured successfully by drug treatment as both the patients have not shown any signs and symptoms of the disease for a period of nearly three years

Case 1 —Had a lesion in the deep muscles of the back One night, while he was travelling in a train and was asleep he had a fall from the bench, striking his back against the floor Although the injury was not of a severe character, a few days after, he began to feel pain of a dull aching character in his back muscles The pain grew worse at night and used to radiate across the other side and to the right iliac region Later on, signs of diffuse inflammation with rise of temperature varying from 99°F to 101°F followed, for a fortnight The inflamed area localized to a head with subcutaneous œdema [illegible]

with the following results —

(1) Stained film examination did not reveal the presence of any pyogenic bacteria

(2) Cultural examination in ordinary media did not show any growth after 72 hours incubation

(3) Animal inoculation test in guinea pig for tuberculosis was made which in its usual course showed negative results

The blood was tested for Wassermann reaction which also gave a negative reaction

Failing to make a positive diagnosis as to the nature of infection by the microscopical and cultural methods of examination of the pus, I had to fall back upon a policy of wait and see in the mean while examination of the discharges daily by the wet film method and inoculation of nutrient broth and maltose agar media was carried out in the expectation of isolating any fungal organism

On the eighth day under the high power of the microscope, by the wet film method, I could find in the pus many large macrophages containing numerous small rod shaped bodies which appeared to me like bits of filaments of a fungus, grouped together in masses inside the phagocytes The bodies could not be stained with the ordinary aniline dyes but they took up aniline gentian violet feebly In the meantime some of the first set of inoculated maltose agar tubes began to show tiny opaque white colonies in them On examination the colonies were found to consist of very fine Gram positive non septate filaments of fungi The inoculated nutrient broth tubes showed the presence of growths like tiny puff balls, woolly in appearance, collecting at the bottom of the tube the supernatant, broth medium remaining quite clear The growths, on examination by the wet film method, showed the true radial arrangement of the fungal filaments of actinomyces A positive diagnosis of actinomycosis in this case was made at this stage of the examination

On continuing the examination of cultures in the broth tubes for another week I could find the fungal filaments undergoing a process of segmentation into small bits exactly like the bacillary forms I noticed inside the large macrophages in the pus films Later on they became smaller still into coccoid *forms* In fact, one could hardly *recognize them, at this stage, as of fungal origin*

From these findings I infer that the fungal filaments of actinomyces may exist in a bacillary or coccoid form inside the large macrophages in the affected tissues and in their discharges and I believe they may be seen in the discharges long before the appearances of any 'grains' or fish roe bodies, which are now regarded as a very characteristic and diagnostic feature of the infection

In fact, at present, we have no sure means of diagnosing early an infection of actinomyces until we can fish out the characteristic 'grains' in the discharges, and I have observed that these grains appear in the discharges at a later stage of the infection, as for instance, in *Case* 2 of my series with lesions in the *right hand* which was an acute case, as it started with cellulitis of the hand after an injury I had occasion to examine the discharges daily for sometime and although the segmented bacillary forms of the filaments in the phagocytes were found in the pus *after the tenth day of the disease*, it was not until the 17th day that I could detect the presence of the tiniest grain and that even, under the high power of the microscope while the larger grains, which could be detected by the naked eye, appeared at a later date

Thus I may remark that the following are some of the noteworthy features of the early diagnosis of a case of actinomycosis —

First There may be a history of injury followed by signs of focal inflammation with intense pain of a lightning character Second The discharges from the affected area, unless secondarily infected, do not reveal the presence of any ordinary pyogenic bacteria, either by the stained film method or by the cultural examination—the inoculated tubes do not show any growth for three or four days

[illegible] ng character in an inflam[illegible] g of any ordinary pyog[illegible] of the discharges *are features which are strongly suggestive of the fungal nature of the infection* This suspicion can easily be confirmed (i) either by continued examination of the inoculated culture media in which colonies of fungi may appear as early as the fifth or sixth day (ii) or by daily examination of the discharges by the wet film method with a view to finding out the bacillary forms of the fungus inside the large macrophages in the pus When these are found present, they can easily be differentiated from ordinary bacteria (1) by staining a smear with ordinary aniline dyes—the fungal filaments will hardly take any stain while the ordinary bacilli take the stain readily, and (2) by growing them in agar media when the ordinary bacteria will grow readily and the fungal filaments of actinomyces will take about five or six days to show any visible growth

When the infection gets a firm footing in the tissues, as is evidenced by the presence of 'grains' in the discharges which can be detected by the naked eye, it becomes difficult and often impossible to eradicate the infection by drug treatment and nothing short of an amputation of the diseased area will give the chance of a radical cure

Of the remaining cases under my observation, the one with the lesion in the cervical and submaxillary glands and the other in the female breast are worthy of mention as they have revealed some important information

The cervical gland case was provisionally diagnosed as of tuberculous origin and the true nature of the infection was only recognized after examining the micro sections of the excised glands The patient gave a history of gradual swelling of his cervical glands There was severe pain all over the

swollen area No history of any injury could be obtained. No definite signs of ulceration of the gums or mucous membrane of the mouth could be detected Teeth were found normal Tonsils looked apparently healthy and of normal size No ulceration was found in the anterior nasal passages In fact no signs of any diseased area could be detected from which the site of inoculation of the infection could be traced The micro section of the excised glands revealed the presence of a typical actinomycotic arrangement of the fungal filaments

I conclude from this case that actinomycotic infection may exist without the least suspicion of it and without showing any lesions in the mucous membrane through which the infection may have passed This case has also shown that actinomyces like tubercle bacilli, can gain entrance into the human body through the lymphatic channels and at the same time without showing any signs of the primary lesion in the mucous membrane—we may here justly compare the post mortem findings in a case of tabes mesenterica—without any visible ulceration in the intestinal mucosa Were it not for the careful examination of the micro sections of the glands, I am confident it would have gone to increase the percentage of the incidence of tuberculosis of cervical glands I may aptly remark here that on more careful examination of the material and tissues from indefinite cases of tuberculosis in various parts of the body we may find out that the incidence of actinomycotic infection in human beings is not so rare as it is thought to be at present

The other case viz actinomycosis of the female breast is no less important owing to the very rare nature of the lesion * The case was provisionally diagnosed as scirrhus cancer of the breast and the true nature of the disease was only recognized when the amputated gland was sent to us for opinion on its micro section The patient had a history of chronic mastitis and ulceration of her right breast for about ten years The gland showed an atrophic condition of its tissues with many chronic ulcers and sinuses discharging offensive pus The breast was found fixed with the chest wall with slightly retracted nipple, but the corresponding lymphatic glands in the axilla were not found enlarged

The micro section of the amputated gland showed the presence of chronic granulation tissue with typical astral arrangement of the fungal filaments here and there

The next case is one with lesions in the right foot The patient was in Sir Frank Connor's ward in the Medical College Hospital for the treatment of Madura foot The whole of his right foot was swollen and indurated and there were many discharging sinuses The disease commenced 12 years ago The first operation performed by Sir Frank Connor revealed the fact that the affected area mainly consisted of very hard fibrous tissue with few irregular cavities containing yellow mucoid flu[illegible]

[illegible] ultimately the leg was amputated

Besides the presence of many actinomycotic grains in the affected area, the micro section of the tissues showed the following changes (1) Numerous plasma cells (2) Presence of a fair number of eosinophile cells (3) A large number of polymorphonuclear leucocytes—(this is evidence of secondary infection with

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* The specimen is in the Medical College Pathological Museum (Series XV 21) As far as I have been able to find out no other case has been recorded in India

pyogenic bacteria) (4) Many large macrophages engulfing a few polymorphonuclear cells and others showing hæmosiderin pigments (5) A few irritation giant cells (6) Signs of general fibrotic changes with many young fibroblasts

As this case remained in the hospital for a long time, I had the opportunity to observe the cultural characters of this fungus in various media, the results of which I may very briefly note down here

The fungus grows in nutrient broth and agar but more readily in maltose agar The description of growth is the same as that previously recorded in my first paper on this subject(1)

On blood agar it grows rather slowly and is not hæmolytic, the growths show pigment after a fortnight In inspissated serum, it grows slowly but the colonies dip down into the substance of the clotted serum which appears to melt down by the proteolytic enzymes which the fungi seem to elaborate as the growth penetrates

In Dorset's egg medium the fungus also grows slowly and shows pigment formation It does not clot milk

The chromogenic property of this red type of actinomyces is also noteworthy The fungus elaborates a kind of pigment, either pink or red or orange It appears that the pigments are elaborated more freely in the presence of some kind of sugar I have noticed the pink red colour develops readily in maltose agar in seven or eight days while the same fungal organism when grown in nutrient broth or nutrient agar remained unpigmented for months even Further when unpigmented colonies from the nutrient broth cultures are transplanted into maltose agar the chromogenic property of the fungi is restored and they develop pigments in the growth, either pink, red or orange in six or seven days This I regard as an interesting finding

The red variety of actinomycotic mycetoma is very rare in India as only a very few cases are in the records In 1860 Vandyke Carter first reported a case of the red type of the fungus Then in 1901 Cornwall reported the second case In 1905 Pelletier described a case of mycetoma with red grains In 1912 Thiroux and Pelletier, in Senegal, reported a few cases of the red variety and named the fungus as *Nocardia indica*, Pelletier Since then, no detailed observation has been made in India on this red variety of fungus

The growths obtained by Pelletier and Thiroux were ruby red from the commencement while with the type under my observation, the growths were at first white and after six or seven days became pinkish red and later on deep red or deep orange

In Pelletier's case the fungal growths in solid media did not dip down into the substance of the medium and so were easily transplanted while with the present type the growths penetrated into the substance of the medium and had to be literally dug out for making subcultures

The white type is not chromogenic, while the red type at first remains unpigmented but the colonies in maltose agar after six or seven days incubation

begin to show colours of variable shades It is in the outer zone of the grains that the pigments are found while the central zone remains unpigmented

The typical clubbing of the terminal filaments of fungi as is met with in the white type is absent in the red type

When uncontaminated grains of the red type are grown in inspissated serum medium the growths go deeper and deeper into the serum by the help of a proteolytic ferment which the fungi apparently elaborate after an incubation of a fortnight the terminal ends of the filaments appear enlarged and form what are known as *arthospores* but the true spore formation has never been observed in any stage of its growth

Biological reactions of the red variety of fungus in sugars —(a) Sugar tubes inoculated with a pure broth culture of the red type when incubated at 37 5° C

The glucose tube showed slight acidity after four days and then became markedly acid but no gas formation has been noticed even after nine weeks incubation Lactose maltose mannite raffinose and salicin all show acid production after two months incubation No gas was found in any one of these tubes The saccharose and dulcite remain unchanged The growths in maltose and mannite showed the red pigment (b) In a similar series of inoculated tubes kept in a cold incubator at 24° C the glucose tubes only showed acid formation after three weeks and the salicin after four weeks No gas was found in either Although the fungus grows in maltose saccharose dulcite and lactose no changes could be seen in these sugar media even after two months incubation

I conclude my paper with many thanks to Sir Frank Connor I M S Professor of Surgery Medical College who not only permitted me to report this rare case of the red type but very kindly helped me with all the necessary materials from his cases for my observation My grateful thanks are also due to Major Shanks I M S for his very kind assistance and suggestions and my sincere thanks are due to my colleagues Dr M N De and Dr D M Chatterji for their kind help in the preparation of macro-specimens and micro sections of the tissues from the different cases for my paper

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post-mortem examination is conducted and smears from the heart and spleen and cultures from the heart blood are made and examined If the material inoculated had been infected with anthrax, one or both the guinea pigs generally die in the course of the third day after the inoculation (about 48 hours or later) and in them lesions such as gelatinous exudate subcutis enlarged spleen sanguineous discharge from nostrils in some cases are generally present In the smears made from the heart and the spleen, anthrax bacilli can be seen in good numbers and this is also further confirmed by the examination of the cultures If the material inoculated is not infected, generally both the guinea pigs remain alive and in those cases where death occurs in one or both of them due to other contaminating organisms death takes place in the course of the second day (in the course of 24 to 48 hours) The lesions usually seen in such cases are an emphysematous condition of the carcass easy peeling off of the skin and no enlargement of the spleen Blood examinations made from the heart, blood and the spleen do not show any anthrax bacilli nor do cultures made from the heart blood

If however both the guinea pigs inoculated died within 24 to 48 hours and if in any of them anthrax bacilli could not be found either in the smears or in cultures it was considered that the guinea pigs might have succumbed to infection from soil organisms in the injected material with which it might have been contaminated before the anthrax spores if there had been any, in the material had time to develop and produce the disease The tests in cases when there was any doubt were repeated

The following statement (Table I) shows the number of samples received in the course of the last official year in the laboratory and the number in which infection with anthrax was discovered The latter works out to a percentage of 27 5 of the samples examined From April 1927 up to the time of writing this paper, i e 22nd November 1927 forty two samples were received, of which eight have been found infected giving a percentage of 19 1 —

TABLE I

Showing the samples of industrial material tested during the year 1926 27

Sample No	Date of receipt	Nature of the sample	Result of test
1	1- 4-26	6 dry goat skins	Free from anthrax
2	"	3 dry salted goat skins and 3 atlas cured goat skins	
3	9- 4-26	6 dry goat skins	*Anthrax*
4		6 dry salted goat skins	Free from anthrax
5	17- 4-26		*Anthrax*

TABLE I—*contd*

Sample No	Date of receipt	Nature of the sample	Result of test
6	26- 4-26	6 dry salted goat skins	Free from anthrax.
7	27- 4-26	6 dry goat skins	"
8	8- 5-26	4 dry salted goat skins	"
9	"	6 dry goat skins	"
10	13- 5-26	1 bundle of hide fleshings	"
11	"	6 dry salted goat skins	"
12	9- 6-26	"	*Anthrax*
13	14- 6-26	6 dry goat skins	Free from anthrax
14	,	"	"
15	27- 6-26	Hide fleshings	"
16	10- 7-26	6 dry goat skins	"
17	28- 7-26	,	,
18	6- 8 26	6 dry salted goat skins	"
19	8- 8-26	6 dry arsenicated goat skins	"
20	20- 8-26	8 dry salted goat skins	*Anthrax*
21	25- 8-26		,
22	28- 8 26	Hide fleshings	Free from anthrax
23	29- 8-26	8 dry salted goat skins	"
24	6-10-26	6 dry salted goat skins	*Anthrax*
25	14-10 26	"	"
26	21-10-26	"	Free from anthrax
27	16-11-26	"	"
28	"	"	"
29	17-11-26	,	"
30	18-11-26	,	"
31	7-12-26	6 python skins ..	"
32	21- 1-27	6 dry salted goat skins	*Anthrax*
33	25- 1-27	6 python skins ..	Free from anthrax
34	29- 1-27	6 dry salted goat skins	*Anthrax*
35	1- 2-27	"	Free from anthrax.

TABLE I—*concld*

Sample No	Date of receipt	Nature of the sample	Result of test
36	8- 2-27	6 dry salted goat skins	*Anthrax*
37	9- 2 2"	,,	Free from anthrax
38	,,	6 tanned skins	,,
39	18- 2 27	6 dry salted goat skins	*Anthrax*
40	,,	,,	,,
41		Hide fleshings	Free from anthrax
42	28- 2 "7	6 dry salted goat sk ns	
43		,,	
44	" 3-""	6 python skins	*Anthrax*
44 (*a*)	12 3 2"		
45	8- 3-""	6 dry salted goat skins	Free from anthrax
46		6 tanned skins	
47	16- 3-"7	6 dry salted goat skins	
48	18 3-27	6 tanned skins	,,
49	21- 3-27	6 python sk ns	,,
50	"3- 3-"7	6 dry salted goat skins	,,

Of the above 20 samples which were all dry goat skins were received fro exporting firm and as the manager of that firm found that ten of such s which were collected from a particular dealer were found infected he appro Mr Ware the then Principal of the College, for advice The matte discussed and Mr Ware advised the manager of the firm as to the desir of having the tannery from which the samples were received inspected b

He agreed to this suggestion and accordingly sent a representative to take me to the tannery

On 3rd March 1927 I visited the tannery with the representative and the agent of the tannery took me round the place and showed me the different sheds where skins are cured The salted skins are first dried in a yard without any pavement in front of the tannery some of the skins are hung on bamboos while others are spread on the floor To prevent undue exposure of the skins to the sun the yard is covered by thatches supported on bamboos As soon as the skins are dried they are transferred to godowns where they are stored and packed in bales ready for delivery There are two such godowns one a smaller one in which the skins cured locally are generally stored and the other a larger one reserved for storing the skins cured locally as well as those imported from mofussil centres For the purposes of this paper I call the shed in which the salted skins are dried as the drying shed and the godowns in which the skins are stored the smaller as shed No 1 and the other shed No 2

Material was collected from four or five different places from each of the sheds and placed in sterile bottles The material collected from the drying shed consisted mostly of sand and mud together with a little wool and salt dropped from the cured skins while those from the two sheds consisted of wool which were found strewn in and around the consignments with mud and dirt

In the examination of this material the main difficulty that was anticipated was the likelihood of the inoculated animals succumbing to infection from the soil organisms with which the material may have been contaminated long before the anthrax spores if any had time to develop This difficulty it was thought would be obviated by repeating the test if necessary with smaller doses of suspensions of the material more highly diluted than in the previous tests Actual weighing of the materials was not adopted and only rough aliquot samples of each were taken and treated in the manner described above Animal inoculations were resorted to at the outset both with the material unexposed and exposed to 80° C but as it was found that the guinea pigs inoculated with material not exposed to heat invariably succumbed within 36 to 48 hours only inoculation of the material exposed to 80° C was made in the subsequent tests

At the first test of these samples both sets of guinea pigs inoculated with the material from shed No 2 and the drying shed died within 24 hours after injection No anthrax bacilli or colonies were detected either in the smears or in cultures As the guinea pigs died within 24 hours after injection further tests were repeated and in these tests also the guinea pigs died in about the same period and no anthrax bacilli could be seen either in the smears nor could any colonies of anthrax be detected in cultures made from the heart blood

As regards the material from shed No 1 both the guinea pigs (one injected with material exposed to heat and the other with material not exposed to heat) died in about 48 hours after injection and *B anthracis* was found not only in the

smears from the heart blood and the spleen but also in cultures from the heart blood from both the guinea pigs In order to confirm this finding further tests were repeated with the material with the positive result

The following Tables II, III, IV show the results of inoculation conducted with the samples of material taken from the different sheds —

TABLE II

Showing the results of tests conducted with the material collected from shed No 1 on 3rd March, 1927

No	Date	No and kind of animal	Short description of the tests employed	Findings	Remarks
I			Aliquot sample of the material was allowed to soak in sterile N S S for an hour and 2 c cs of the fluid were injected sub cutaneously into a guinea pig Then the soaked material was exposed to 80° C for 20 minutes and 2 c cs of the cooled fluid were then injected into another guinea pig		
	5-3 27	G P 154	2 c cs of the not heated material	Died in 49 hours Smears and cul tures positive to anthrax	*Anthrax*
		G P 155	2 c cs of the heated material	Died in 47 hours Smears and cul tures positive	
II	14 3 27	G P 174	2 c cs of the heated material (Prepared again as in the first test)	Died in 27 hours Smears and cul tures negative to anthrax	Probably died before an thrax could develop Inconclusive
		G P 175	"	Died in 18 hours Smears and cul tures negative to anthrax	
III	18-3-27	G P 181	2 c cs of the heated material. (Prepared as in the previous tests)	Died in 24 hours Very few bacilli simulating anthrax in the smears from the spleen could be detected Cul tures negative to anthrax	

TABLE II—*contd.*

No	Date	No and kind of animal	Short description of the tests employed	Findings	Remarks
IV	23-3 27	G P 187	1 c c of the heated material	Died after about 60 hours Smears and cultures positive to anthrax	*Anthrax*
		G P 195	Scarified with the heart-blood of G P 187	Died in 32 hours Smears and cultures positive to anthrax	

TABLE III

Showing the results of tests conducted with the material collected from shed No 2 on 3rd March, 1927

No	Date	No and kind of animal	Short description of the tests employed	Findings	Remarks
I			Aliquot sample of the material was soaked in N S S for an hour and 2 c cs of the fluid were injected subcutaneously into a guinea pig Then the soaked material was exposed to 80° C for 20 minutes and 2 c cs of the cooled fluid were injected into another guinea pig		.
	5-3-27	G P 156	2 c cs of the not heated material	Died in 24 hours Smears and cultures negative to anthrax	Probably died before anthrax could develop
		G P 157	2 c cs of the heated material	"	Inconclusive
II	27-3 27	G P 196	1 c c of the heated material (Prepared again as in the previous test)	Died in 20 hours Smears and cultures were negative to anthrax.	"

TABLE IV.

Showing the results of tests conducted with the material collected from the 'drying shed' on 3rd March, 1927

No	Date	No and kind of animal	Short description of the tests employed	Findings	Remarks
I		..	Aliquot sample of the material was soaked in N S S for an hour and then 2 c cs of the fluid were injected subcutaneously into a guinea pig The soaked material was then exposed to 80° C for 20 minutes and 2 c cs of the cooled fluid were injected into another guinea pig	.	..
	5-3-27	G P 158	2 c cs of the not heated material	Died in 24 hours Smears and cultures negative to anthrax	Probably died before anthrax could develop
		G P 159	2 c cs of the heated material	Died in 27 hours Smears and cultures negative to anthrax	Inconclusive
II	27-3 27	G P 197	1 c c of the heated material (prepared as in the previous test)	Died in 23 hours Smears and cultures negative to anthrax	"
	28-3-27	G P 200	Scarified with the heart blood of G P 197	Alive	

As it was found from the above results that shed No 1 was definitely infected and as I thought that this finding would be a very important one both from the industrial and public health point of view, I made a request to the Principal of the College to make arrangements for my re visiting the tannery for the purpose of collecting fresh material for further examination and confirmation of the above results

On 25th May 1927, I visited the tannery in the company of the same representative, and as before collected material from the same three sheds in sterile bottles using separate sterile spoons for collecting each sample These materials were put to the same test but, in view of the results obtained during the first test, dilutions were made far higher than those used on the previous occasions and the quantities injected were also only half of what was used in the previous tests

The results of the tests are tabulated below in Tables V, VI, and VII —

TABLE V

Showing the results of tests conducted with the material collected from shed No 1 on 25th May, 1927

No	Date	No and kind of animal	Short description of the tests employed	Findings	Remarks
I	21-6-27	G P 287	3 grammes of the material were soaked in 60 c cs of N S S for an hour exposed to 80° C for 20 minutes Then 1 c c of the cooked fluid was injected subcutaneously	Died between the 50th and 65th hour Smears and cultures were positive to anthrax	Anthrax
II	29-6-27	G P 300	3½ grammes of the material soaked in 70 c cs of N S S for an hour and exposed to 80° C for 20 minutes Then 1 c c of the cooked fluid was injected subcutaneously	Died in 45 hours Smears and cultures were negative to anthrax	Probably died before any anthrax bacilli could develop

TABLE VI

Showing the results of tests conducted with the material collected from shed No 2 on 25th May, 1927

No	Date	No and kind of animal	Short description of the tests employed	Findings	Remarks
I	22-6 27	G P 288	5 grammes of the material were soaked in 100 c cs of N S S for an hour and exposed to 80° C for 20 minutes Then 1 c c of the cooled fluid was injected subcutaneously	Died in 26 hours Smears and cultures were negative to anthrax	Probably died before anthrax bacilli could develop
II	25-6-27	G P 291	1 c c of the heated material (Prepared again as in the previous test)	Died in 43 hours Smears and cultures were positive to anthrax	Anthrax

TABLE VI—*concld*

No	Date	No and kind of animal	Short description of the tests employed	Findings	Remarks
III	30–6–27	G P 301	5½ grammes of the material were soaked in 110 c cs of *N S S* and treated as in the previous tests Then 1 c c of the cooled fluid was injected subcutaneously	Died between the 54th and 67th hour Smears and cultures were positive to anthrax	Anthrax
IV	7–7–27	Goat 312	Injected subcutaneously with 1 c c of broth culture of 48 hours' duration of anthrax bacilli isolated from the material collected from shed No 2 through G P 301	Died in 48 hours Smears and cultures from the peripheral blood were positive to anthrax	,,

TABLE VII

Showing the results of tests conducted with the material collected from the 'drying shed' on 25th May, 1927

No	Date	No and kind of animal	Short description of the tests employed	Findings	Remarks
I	22–6–27	G P 289	5 grammes of the material were soaked in 100 c cs of N S S for an hour and exposed to 80° C for 20 minutes Then 1 c c of the cooled fluid was injected subcutaneously	Died between the 50th and 63rd hour Smears and cultures positive to anthrax	*Anthrax*
II	30–6–27	G P 304	5½ grammes of the material were soaked in 105 c cs of N S S for an hour and exposed to 80° C for 20 minutes Then 1 c c of the cooled fluid was injected subcutaneously	Died in 70 hours Cultures and smears positive to anthrax	,,

As a further confirmation of the above result, 1 c c of 48 hours' broth culture isolated from the material collected from shed No 2 and passed through the

guinea pig No 301 was injected subcutaneously into a goat and this animal died within 48 hours after inoculation (*vide* Table VI) Anthrax bacilli were found in the smears and also in cultures made from the peripheral blood

From the information I could gather from the representatives of the firms concerned in the export of hides and skins I find that most of the goat skins obtained for export are collected from slaughter houses and it is very unlikely that any of the animals or at least any perceptible numbers would have been harbouring the infection at the time of the slaughter and if there had been any they would have been detected either before or after slaughter by persons responsible for the inspection of slaughter houses At the same time it is also unlikely that of the skins collected from dead animals many would have been those of the animals which had died of anthrax and if that were the case the attention of the staff either of the Veterinary or Revenue Department would have been drawn to such mortality No such high mortality from anthrax has been returned in goats as far as the Madras Presidency is concerned in the course of the last year

It would therefore, appear that the chances of the skins being collected directly from anthrax infected animals cannot be frequent and the infection noticed in the samples examined must I think have occurred in the tannery This view derives additional support from the fact that infection has been discovered even in the python skins which have been cured likewise but samples of which were received from other exporting firms The question of prevention of infection of anthrax in the industrial materials would therefore appear to resolve itself into one of prevention of contamination of the materials in the tanneries which from import of any anthrax infected skins into them at any time might be harbouring the spores and thus prove to be a perennial source of infection Inspection of some more tanneries and godowns where the skins are cured and stored and examination of materials collected therefrom is deemed highly desirable and if it is found that they are infected likewise, it may be possible to minimize the incidence of the percentage of infection in the industrial materials by adopting a proper system of examination and disinfection of tanneries and godowns from time to time

REFERENCE

Proceedings of the Second Meeting of Veterinary Officers in India Calcutta 19-3 Superintendent Government Printing Calcutta pp 47—49

DISCUSSION

Mr J T Edwards (United Provinces) The subject now brought up by Mr Krishnamurti threatened to become a very important one a few years ago when as the result of representations made by the Bradford wool sorters a special sub committee of the League of Nations was constituted to decide what measures should be recommended to Governments to prevent danger of importation of anthrax with wool hides and hair The Home Government also took up the matter seriously, and a monumental report was drawn up by the late Prof Delepine on the

technicalities of the subject, including the methods of treating infected material so as to render them innocuous The methods available to combat the danger of importation comprised either the erection of expensive installations for the treatment of material that was probably infected at ports of import or export (and a large experimental installation was set up at Liverpool for the purpose) or the institution of adequate measures by veterinary police in infected countries to control the incidence of infection among animals Action has been kept in abeyance since 1923 largely because experts were not entirely agreed as to which measures the more suitable and also because of the expense of the measures in either case

Anthrax is not uncommon among animals in India, in fact it is much more common than the official records would indicate, but it is curious that the disease is nearly always sporadic in its occurrence, and shows little tendency to assume the form of large epizootics, as in South Africa and Argentina Strains of anthrax bacilli of very low pathogenicity are not uncommon in India and it is not unlikely that the temperature conditions are often suitable for the propagation of the organism in vegetative form as a saprophyte outside the animal body and perhaps meanwhile the organism becomes degraded in violence It is difficult to assess the value of the technique of examination described by Mr Krishnamurti without reading his paper

Dr G Panja (Calcutta) One speaker (Mr Edwards) has pointed out that *B anthracis* multiplies outside the body, if a suitable body temperature alone is obtained Hence the virulence of the bacillus is decreased and there is no possibility of infection of mankind by wool, hides, etc I have kept a dried culture of the bacillus on absolutely dry media in the suitable temperature of the incubator, but I have failed to observe any multiplication, that is, only spores have been found and no bacilli

SECTION IV.

TYPHUS-LIKE DISEASES, LEPTOSPIRÆ, ETC

TYPHUS LIKE FEVERS CONVEYED BY TICKS

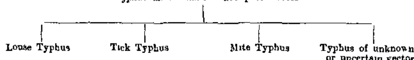
BY

LIEUT COL J W D MEGAW C I E I M S
Director School of Tropical Medicine and Hygiene Calcutta

FRIDAY DEC 9TH 2 TO 4

THERE are two forms of fever which have so obvious a resemblance to *Typhus exanthematicus* that the clinician would have little hesitation in placing them in the typhus group These are the Rocky Mountain spotted fever and the Japanese River fever or Tsutsugamushi In the case of the Rocky Mountain fever the pathology has been worked out very thoroughly by Ricketts and Wolbach whose investigations have shown that the pathology of typhus and Rocky Mountain fever is remarkably similar

The pathology of the Japanese disease has not been worked out so completely but the recent work of Nagayo and his colleagues points to its being essentially similar to that of the other two diseases Even before the reports of the Japanese workers appeared I was so struck by the broad clinical resemblances of all three typhus like fevers that I suggested the following classification —



This classification does not pretend to be final but it has the following advantages —

(1) The name 'typhus' at once suggests to the clinician a self limited fever with a peculiar rash, and when such a fever is encountered it is helpful to the medical man that there should exist a suggestion that the disease belongs to the main typhus group

(2) The use of the name of the vector forms a second helpful suggestion The doctor's attention will be directed to the epidemiological conditions under which the disease occurs

(3) When we have complete knowledge of the vectors concerned with the *conveyance of typhus the classification will* become a complete and scientific terminology with such modifications as may prove to be necessary if still other arthropods are found to be implicated The drawbacks of the existing names are obvious To apply the name of a place to a disease is to suggest that the disease has a strictly limited distribution and medical men will not think of making a diagnosis of Rocky Mountain fever or Japanese River fever when they come across a case of disease in a far distant locality Such names introduce an inhibition in the mind of the doctor and they automatically become entirely unsuitable when the diseases turn out to have a wider distribution than was at first believed

For these reasons I do not hesitate to recommend a change in nomenclature in spite of the fact that changes in names are often confusing and should only be made when there are excellent reasons for the action

The application of place names to diseases has already caused much confusion for example such names as 'Malta Fever,' 'Delhi Boil' 'Chitral Three Days' Fever,' 'Calcutta Seven Days' Fever,' 'Arcon Fever," etc There will be few advocates of such a name as 'Spotted Fever' because this has already been applied to several fevers and many fevers are associated with a spotty rash

The mite borne typhus like fever has already been shown to exist in several places outside of Japan and a fever which has not been differentiated from the Rocky Mountain fever probably occurs in many parts of the world

In the case of mite typhus the frequent occurrence of a local sore with local lymphangitis is very helpful in the diagnosis in the case of tick typhus we have *no such aids in the recognition of the disease, indeed* we are *often left* in grave doubts as to whether the cases are of louse typhus or tick typhus The rash perhaps may prove to be a reliable guide but such variations occur in the rash of louse typhus and tick typhus that it would be unsafe to depend on the distri*bution or characters of the rash for a differential diagnosis* The Wilson Weil Felix reaction has proved somewhat equivocal hitherto though it may eventually become a safe guide The evidence of person to person infection by lice is often so clear that no difficulty arises but there have been numerous sporadic cases in which there was *no satisfactory evidence as to the vector*

In the tick borne type of the disease the tick may remain *in situ* as evidence *of its guilt but many cases occur which are otherwise indistinguishable* from these although no tick has been observed, the most probable explanation being that the tick has bitten and dropped off *leaving no clue to its action*

The severity of the cases does not help at all in diagnosis The mortality in tick typhus and mite typhus varies from two or three per cent up to 50 per cent or over, and the severity of louse typhus also varies greatly in different epidemics In this paper an attempt will be made to give *a brief summary of the* evidence for the existence of tick borne typhus fever in various localities in India and other parts of the world

Apart from the Rocky Mountain fever which has been proved to be conveyed from rodents to man by a tick—*Dermacentor andersoni*—the first definite suggestion that a typhus like fever was conveyed by ticks appeared in a note by me on an attack of fever from which I suffered myself The attack of fever occurred in July 1916, it began 20 days after the bite by an unidentified tick which bit me in a forest at a distance of about one and a half miles from Sat Tal in the Kumaon Himalayas The resulting fever was definitely typhus like with characteristic spotty eruption which appeared on the fifth day and left a staining which lasted for more than a month

There was a striking resemblance between my illness and the accounts of Rocky Mountain fever, so that, taking everything into account it seemed probable that the tick which had bitten me was responsible for the attack of fever At this time I was informed that Col McKechnie I M S, had made an enquiry in 1913 into the fevers of the locality in which I had been bitten by the tick and I was able to secure a copy of his unpublished report The cases reported by McKechnie were very similar to my own and it was remarkable that McKechnie had set out on his enquiry with the idea that he was dealing with a typhoid group fever but was forced to the conclusion that the disease was typhus The idea of tick transmission did not occur to him though he entertained the possibility of the disease being the same as the Rocky Mountain fever My attention was also directed to a report by Capt McNaught in 1911 in the *R A M C Journal* on 'Paratyphoid Fever in South Africa' The clinical features of McNaught's cases and the conditions under which they occurred point rather strongly to their being of the same type as the Rocky Mountain fever and the Kumaon fever There is a significant reference in McNaught's paper to a suggestion by Col Maher, R A M C, that ticks might be concerned in the causation of this fever though McNaught merely referred to this in passing and did not seem to attach any importance to it

Since the date of my first paper—January 1917—a large dossier of evidence has accumulated which shows that a fever of the same general type is frequent in various parts of India, Nigeria, the Federated Malaya States the Eastern States of North America, Australia, East Africa, and elsewhere The problem has already been discussed by me in several papers in the *Indian Medical Gazette*, but here I will only deal with the evidence which points to tick transmission

The following cases are those in which a clear association with tick bite has been ascertained, unfortunately in no case has the tick been captured and identified, as the possibility of its being a disease vector had not been considered by any of the persons who were bitten —

(1) A European lady in Hyderabad, Deccan, seen by Lieut Col Sprawson, I M S, in September 1917 This lady had an attack of fever very similar to mine, the fever began about a fortnight or three weeks after she was bitten by a tick Col Sprawson had seen me during my illness and was at once struck by the similarity of the rashes he therefore made enquiries about ticks, otherwise it is pretty certain that the information would not have been elicited Col Sprawson had seen louse typhus in Mesopotamia and was of opinion that the rash in his Hyderabad case and in mine was different from that of louse typhus in being more prominent on the extremities and face, pinker

and with less skin mottling In his patient lice could be excluded with reasonable certainty and there was no evidence of the occurrence of other cases from which infection could have been conveyed

(2) A case reported by Dr B M Mukerji from Narsingunge, Dacca in which typhus fever was diagnosed by Col Anderson, I M S The patient was a well to do European who had been bitten by a tick seven days before the onset No lice could be found and no other possible source of infection could be discovered than the tick bite

(3) A case shown to me by Lieut Col Waters I M S The patient was a European male who had found a tick crawling on his body about 12 days before the onset of the fever, while he was living in Akyab in Burma Lice were excluded and the Widal and Wilson Weil Felix tests were negative

(4) and (5) These two cases are of very special significance as they gave rise to great difficulties of diagnosis owing to the fact that the doctors who were at first in charge had not heard of the existence of a typhus like fever conveyed by ticks I am indebted for the details of the cases to Major Boyd I M S, Dr Brandon and Lieut Col Barnardo I M S Both patients were well to do Europeans of Calcutta they were members of a small party who went into a camp near Balaghat in the Central Provinces of India in the Christmas week of 1923 24 Tents were used and these were pitched on a site which had never been used before Lice were excluded and the conditions of life were such as to make chance louse infection exceedingly improbable The general type of fever and rash were the same in both cases, a macular and petechial rash occurred all over the body leaving stained spots for more than six weeks In one case a tick was found fastened on the scrotum eight days before the onset in the other a tick was found on the umbilicus, it was engorged with blood and was discovered two days after the onset, this had certainly fastened itself on the patient several days previously while he was still in the camp The Widal was negative in both and in the one, in which a Weil Felix test was carried out this was negative

(6) A case of typhus like fever following tick bite is reported by R R Spencer in the U S Public health Report of 5th November 1926 The wife of a butcher in Norfolk, Virginia, was bitten by a tick from a calf hide which came from north Carolina or Virginia There was redness, swelling and a small ulcer at each site of the tick bite Ten days after the bite fever set in, the course of this and the rash appear from the report to be exactly similar to those recorded from India The Widal and Weil Felix reactions were negative and the guinea pig inoculation was doubtfully positive with 13 days' incubation but sub inoculations into guinea pigs and a monkey were quite negative It is [illegible] the

[illegible]

[illegible] h

started six days after a bite by a tick in the Darjeeling district Full details are not yet available

These cases taken together constitute very strong evidence that there is a tick borne typhus like fever in localities far distant from the Rocky Mountain fever area

There are good many other cases in India in which there was strong presumptive evidence of an antecedent tick bite, but I have only included the cases in which the association has been definitely proved

The next point to be considered is whether the large number of other cases of fever of a similar clinical type belong to the same group Many of these cases have been recorded and discussed by me already so I do not propose to enter into details regarding them These and a number of hitherto unreported cases will be dealt with in a paper which is in preparation

The groups of cases which appear to bear most directly on the problem are —

(1) The cases described by McNaught in South Africa in which Col Maher suspected ticks as the vectors

(2) The cases described by McKechnie in 1913 in Bhim Tal and Sat Tal which were regarded by him as typhus and which occurred in the very locality in which I contracted my attack It seems probable from all the available evidence that this locality is an endemic focus of the disease a large proportion of all the Europeans who have resided in the area have suffered from a typhus like fever

(3) The group of 18 cases in Nigeria in 1920 described by Wynne Davies and Johnson as a Twelve day Fever of the Dengue Group' discussed by me in the *Indian Medical Gazette* of October 1921

(4) The group of nine cases which occurred among 2 000 soldiers in two camps near Saugor in Central India in February 1924 observed by Major Shettle, I M S Dr D N Roy and myself and described in the *Indian Medical Gazette* in February 1925

(5) The Pseudo or Para Typhus of the Kenya Colony described by Anderson in the *Kenya Medical Journal* of May 1925 In this paper there is a reference to a similar disease observed by J A Mitchell of Cape Town

(6) 'Tropical Typhus' in the Malay States—122 cases with five deaths discussed by Dr William Fletcher in Bulletin No 2 of the Institute for Medical Research Kuala Lumpur in 1926 and reported at the last Congress of the Far Eastern Association of Tropical Medicine

There are several other records of cases which must be considered in a detailed examination of the problem but these have been deliberately omitted as their consideration would take too long

The features common to the six groups of cases are —

(1) All of the observers describe a typhus like fever with characteristic rash and low mortality

(2) All the cases occurred under conditions in which person to person communication by lice could be excluded with reasonable certainty The cases were sporadic they occurred among persons living under the conditions which prevail in the open country or forest

(3) Several attempts to inoculate guinea pigs and monkeys have failed

(4) With the striking exception of Fletcher s cases the Wilson Weil Felix reaction has always been negative except in a few cases which reacted in dilutions of 1 80 and under

(5) Clinically these cases all show a remarkable resemblance to the cases in which an association with tick bite has been established

We are on safe ground when we assert that a typhus like fever occurs in many parts of the world under conditions which make the transfer of the disease by an arthropod vector from an animal reservoir the most likely mode of transmission

Which is the most probable vector ? Ticks and mites are the only known vectors of a typhus like fever The mite borne disease is described as having a local sore at the site of infection a local lymphangitis and lymphadenitis Under these circumstances the mite can be regarded as unlikely to be the vector of the disease in question In the case of the tick the points are (1) the clinical

manifestations and epidemiology closely resemble those of a disease known to be conveyed by ticks viz Rocky Mountain fever

(2) In a number of well authenticated cases of a similar fever in some of the localities concerned a tick has been proved to have bitten the patient within the probable period of incubation

(3) All of the outbreaks have occurred under conditions in which tick conveyance from an animal reservoir was likely to have occurred There is thus a considerable amount of *prima facie* evidence that all of these cases may have been caused by tick bite

The difficulties which arise are —

Is it likely that so many people could have been bitten by ticks without being aware of the fact or as an alternative without giving any information on the subject ?

The entomologists must be consulted on this point but there are several cases in which the history of a tick bite has been elicited only by direct enquiry there are cases in which the tick has only been found when a search has been made although in some of these the tick must have remained *in situ* for several days before being noticed There are places in which the inhabitants have asserted that human beings are not bitten by ticks in that locality, but personal observation has shown that ticks do bite quite frequently in these very localities My personal experience is that the tick is often elusive its bite may be absolutely painless and no trace of its attack may be left It is therefore quite possible for the tick to be overlooked unless it is carefully sought for

The next point is do these isolated groups of cases represent one or several forms of disease and are they the same as the Rocky Mountain fever ? Clinically they cannot easily be distinguished but there has been a remarkable failure to inoculate guinea pigs with the blood of affected persons, whereas in Rocky Mountain fever such inoculation is strikingly easy I have recently had an opportunity of discussing this point with Dr Wolbach whose magnificent reports on typhus and Rocky Mountain fever are so well known, he said that he would be surprised if a disease similar to Rocky Mountain fever were not readily inoculable to guinea pigs He was not dogmatic in stating that all the forms of Rocky Mountain fever are readily inoculable but he believed this to be the case

Another interesting point arises in connection with the Wilson Weil Felix test This has been uniformly negative in high dilutions except in Dr Fletcher s cases which present the interesting feature that some of his cases reacted strongly to a non indol producing strain of Proteus X 19, while they were negative to an indol producing strain and the rest of the cases reacted to the indol producing strain but were negative to the other strain It is evident that if only one strain had been used his cases would have been sharply divided into two groups the one being Weil Felix positive and the other Weil Felix negative The serological reactions therefore, need much further study before we can rely on them for the differentiation of the cases Possibly the same may hold true of animal inoculation

The relationship between tick typhus and Brill's disease is an interesting point McNaught McKechnie and myself were all inclined to think that our cases might fall into the Brill group but, when it was reported that Brill's disease had been proved to be mild typhus of a sporadic type and when I considered that Brill's disease occurred only in large centres like New York, I had to agree with the conclusion that it fell into the louse typhus group Maxcy and other American workers are now engaged in throwing grave doubts on the view that Brill's disease is conveyed by lice and are looking for some other arthropod vector and for a possible animal host

Maxcy's recent study of endemic typhus (Brill's disease) in the South Eastern United States deals with this question The disease which he has studied occurred almost entirely in towns or cities in the South of Alabama and in the city of Savannah The Weil Felix reaction was almost uniformly positive in the *cases and successful transmission to guinea pigs and monkeys has been reported* In these respects the disease would appear to be quite different from the tick borne typhus of India

There is, however, reason to suspend judgment as to the significance of animal transmissibility and the Weil Felix reaction and, although at first sight a disease which occurs in towns is unlikely to be the same as a disease of people living in the open country, we must not forget that many of the residents of the towns in America make weekly excursions into the country and on these occasions they are likely to be brought into close association with the life of the wilds including ticks I would therefore suggest that the tick should be considered as a possibility even in connection with Brill's disease

The Mossman fever the typhus-like fever of Adelaide, the Fièvre Boutonneuse of Tunis and some other problematic typhus like fevers need consideration, but what has been said ought to convince my hearers that the typhus group of fevers constitutes a fascinating problem which is far from being solved One interesting side issue is the question as to whether tick typhus and louse borne typhus may not have a common ancestry Human diseases are often transmitted to lower animals and vice versa and it is quite possible that the differences between the virus of louse typhus and tick typhus may be accounted for by modifications occurring in consequence of a transfer through different animal hosts So far as I know attempts have not been made to transfer typhus to animals by ticks or tick typhus by lice it would be interesting to carry out these experiments

Summary

[illegible] has a wide distribution [illegible] to man by ticks Tick [illegible] nce is still lacking as to whether tick typhus of India is identical with Rocky Mountain fever but it almost certainly belongs to the same disease group and the name tick typhus will probably be the most suitable for all the typhus like fevers which are conveyed by ticks

Other arthropods besides lice, ticks and mites may
fevers of the typhus group, but evidence of this is

DISCUSSION.

Dr O Schobl (Philippine Islands) Considered t
tiation between the disease under discussion and the
temperature curve and blood picture of monkeys inocu

Major T O Thompson, R A M C (B India)
whether he knew of the recent outbreak in October of
Body guard at Dehra Dun There were seven cases o
source of infection from any known centre of louse bor

There was a possibel source of tick infection in tha
habit of grazing their horses in a valley known to be i
asserted that they were never attacked by these ticks
type with two deaths, but the Weil Felix reaction nev
low for true typhus The outbreak was puzzling and
Col Megaw could throw light on it The details will
officer concerned

Dr C Strickland (Bengal) Col Megaw mention
apparently acquired an infection of pseudo-typhus w
Darjeeling district I am very interested in this as p
locality to which I know he is accustomed to go and w
hares, as I propose to suggest in my paper to be read s

On another point, I think Col Megaw overvalues
lymphatic lesions as evidence of the infection not bei
others have shown that the virus of Japanese Riv
cutaneously does produce lymphatic lesions while su
lesions may, therefore, depend on the length of the pr

Col J W D Megaw, I M S (Bengal) Replyin
cases dealt with in the paper appeared to be very closel
spotted fever they might be identical but this point h

Major Thompson s group of cases in Dehra Dun n
typhus group and if fuller information were supplied
glad to express an opinion Dr Strickland's suggest
from the bite of mites, although no local manifestation
interesting possibilities but the strongest point in
considerable number of the patients had been bitt
incubation period The other cases in which ticks ha
close clinical resemblance to those in which ticks had a

A PSEUDOTYPHUS EPIDEMIC IN SOUTHERN QUEENSLAND AND ITS ÆTIOLOGICAL BEARING UPON CASES IN INDIA.

BY

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THIS epidemic is reported because of its possible bearing on the ætiology of the clinically similar condition in the Indian Peninsula to which attention has recently been drawn by Lieut Col Megaw (1917 *et seq*)

The cases in the epidemic referred to were kindly shown to me by Dr Falkiner of Toowoomba while I was on leave in Queensland, and the most interesting point about them was that while in their diagnosis typhus had come under the anvil of discussion this condition had been ruled out because of the almost exclusive incidence of the cases in the rural areas the cleanliness and freedom from lice of the patients, the apparent lack of communicability from person to person in infected houses and the defervescence by lysis.

Through the kindness of Dr Falkiner, and the Resident Medical Officer of Toowoomba Hospital, I saw a number of the patients and am thus enabled to point out the similarities or otherwise to the Indian type As, however, not many of them had been admitted into hospital before the fifth day, one must rely largely upon the statements of the patients themselves for any knowledge of the earlier signs of the illness

At the onset of this then, there was neither sore nor ulcer, nor lymphatic symptom such as occurs in 'mite typhus' (Japanese River fever),* nor sore throat, nor bowel trouble The first symptoms were headache, langour and drowsiness, these being followed by suffusion of the conjunctivæ, and the tongue soon becomes very dirty as in typhoid It was the furred tongue and remittent temperature that had suggested the diagnosis of typhoid, though bowel symptoms at no time had appeared and Widal's reaction was negative †

* Professor Cleland says in a letter to me regarding the corresponding Adelaide cases, 'None of us has ever met anything suggesting a primary sore or ulcer with lymphatic inflammation '

† It was thought that the material then available for the reaction was possibly not satisfactory Weil Felix had not been carried out

The temperature remained up for about a fortnight and came down by lysis the charts being similar to those figured by Megaw Rash was not often noticed before the fifth or sixth day but persisted throughout the second week of the illness and was seen chiefly on the arm leg back and chest no staining after defervescence In one case which I saw, and that the most '*mental*' *the typhus odour was very* marked *

The above short description should suffice to show the clinical identity of the condition with the Indian illness named by Megaw tick typhus The epidemic mortality apparently had been nil

Ætiology of the Queensland Cases

With regard to the ætiology of the epidemic the first points to note are those that had been considered evidence against the cases being louse typhus viz the 'dropping' nature of their incidence the apparent freedom of the patients from lice, and the occurrence of the epidemic in the rural areas most of the patients being *farmers and farm hands moreover, most of them were males which would not* have been the case in a louse transmitted epidemic The general medical opinion regarding the cases seems to have been that there had appeared for the first time an illness which, unlike jail *typhus,* was *correlated with a plague of mice then* over running the country

The incidence of the cases indeed not only weighed heavily against any idea of louse transmission but also of causation by any other domestic agency, whether a parasite or a medium such as infected food, like weevily flour which had I believe, been thought of

Indeed any ecto parasitic explanation was difficult as in none of the Queensland cases had there been any history of an 'insect' bite

However *it is now proposed to discuss two hypotheses suggesting an ecto* parasitic origin of the disease which would be compatible with the main facts of the epidemic as outlined above —(1) the transmission of the disease to man from an animal reservoir by a non domestic parasite facultative to both, and (2) the transmission direct from man to man by a non domestic arthropod e g by a 'bush' tick

A priori of these two hypotheses the ætiology of the typhus like fevers in other countries indicates the former as the more likely, in 'Rocky Mountain fever' the virus subsists in certain rodent reservoirs in 'Japanese River fever' the reservoir is a field mouse and the Adelaide cases reported by Hone (1922 *et seq*) had a noticeable association with rats grocers' shops and stores of wheat so much so that the illness earned the popular title of the 'wheat disease'

* Wheatland's paper (1926) on the pathology gives more details

(I) THE POSSIBILITY OF TRANSMISSION TO MAN FROM AN ANIMAL RESERVOIR.

(a) The mouse

In the Queensland cases there was cogent direct evidence to the effect that the mouse was the culprit, as has been mentioned Both public and professional opinion was very decided on there being some connection between the epidemic and the coincident plague of ' mice '* and this hypothesis was certainly on all fours with the farmers being those chiefly affected It was indeed suggestive that a similar epidemic had never occurred within the memory of man until a mouse plague had visited the country

With this in view and to study the mouse ecto parasites I obtained from Queensland through the kindness of Dr Falkiner 131 of the mice and they were all as kindly advised by Lieut Col Sewell, I M S , of the Indian Museum, and by Mr Hinton of the British Museum, *Mus musculus*

The world ecto parasites of the mouse as far as recorded are as follows —

(1) *Ticks* —Mr Warburton kindly informs me in a letter as far as I know all the ticks received from the *Muridæ* belong to *Ixodes* ' Those I know of are —

Ixodes ricinus	recorded	from	mice	in	America
I angustus	,	,,	,,	,,	Canada
I niteus		,,	,	,,	Christmas Island
I arvicolæ n sp			,	,,	Cambridge

None of these *Ixodes* are Australian and it will be noticed that *Ixodus holoyclus*, the common Australian bush tick is not mentioned Nor does Ferguson (1924) who has recently reported on this species give the mouse as a host he moreover, says that he has not received this tick (excepting from one locality), from the Australian highlands and that was the site of the epidemic in question, whereas it is widespread in the coastal region

Mr Fielding (1927) gives as additional to the above *I fecialis* found on *Mus* sp

Mr S Hirst of the British Museum informs me by letter that ' Quite a number of ticks have been recorded (from rodents), but none of them seem to be specially addicted to *domestic* rodents ' and Professor Cleland in a letter with regard to the Adelaide cases says ' ticks are absolutely out of the question in connection with the transmission

The evidence then that ticks are the carriers from mouse to man in the Queensland cases is negative

(2) *Mites* other than ticks, may have been concerned The absence of a primary sore or any lymphatic affection such as is common in the ' mite ' carried Japanese River fever might be considered presumptive evidence against ' mites '

* Professor Wood-Jones of Adelaide University tells me that such a mouse plague sometimes declines by the mice becoming sickly and dying off May they then be suffering from an epizoötic due to exaltation of virulence of a typhus virus ?

being the vectors in the Australian cases but Nagayo with others in Japan have reported that *subcutaneous* inoculation of the virus does not give rise to any local or lymphatic reaction while *intracutaneous* inoculation does so the natural deduction from this being that a mite with a short proboscis produces a primary sore while one with a long proboscis does not On this hypothesis the Australian cases might have been carried by a species of mouse mite with a long proboscis *

A point in favour of these mites being the vectors rather than ticks is that the bites of such small creatures would probably pass unnoticed oftener than tick bites, a point which would account for the fact that in the cases under review there had been no history of any bite by an arthropod

I have previously obtained only one species of these mites from mice (*M musculus*) viz *Holostaspis* sp (identified by Mr S Hirst) these having been taken in Calcutta but from the 131 mice which Dr Falkiner kindly sent me from Queensland five 'mites' were taken, they were, so Mr Hirst tells me, of a new species of *Lælaps, L australensis* a Gamasid and therefore with a comparatively long proboscis which would on the above hypothesis not produce any local lesion at the site of the bite Possibly it is this species which will be found to be the vector of Queensland *pseudotyphus*

(3) *Fleas —Ctenopsylla musculi* the common mouse flea is not known to bite man but in view *of Dr* Fabian Hirst's finding that X *astia* will bite man under certain special conditions e g cold *C musculi* conceivably may do so On the 131 mice received from Queensland there were 190 specimens of this flea

(*b*) Possible animal reservoirs other than the mouse

Another rodent or another order of animal may constitute a reservoir and harbour ecto parasites which bite man

1 *Ixodes holocyclus* —From the point of view of the mouse being the reservoir the possibility of a tick being the intermediate host has already been considered, and certain of the evidence then adduced against *I holocyclus* being the vector may be brought forward against it being the vector under any circumstances However the species (the common Australian 'bush tick') must here be shortly reconsidered *in view of its common habit of biting man and its catholic* tastes towards lower animals, any of which may possibly be a reservoir Nuttall and Warburton *loc cit*, give as its hosts in Australia the sheep calf dog marsupial

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* Nuttall (1911) has reported cases of tick bite in which the inguinal glands became enlarged Therefore the cases of pseudotyphus noted by Megaw in which there was femoral adenitis without any primary cutaneous lesion may have also been caused by a tick or another mite and the fact that in these cases the glandular enlargement was in the leg rather points to the tick or mite being the carrier rather than a flying creature bug or louse

Wheatland (1924) has also described cases of a 21 day scrub land fever with enlargement of the glands, while in 'Sarina fever' among the sugar cane cutters there is sometimes glandular enlargement, and Cilento (1923) talks of the epidemic glandular fever of Queensland

tree shrew (*Phascogale penicillata*) and *Macropus* sp, while Ferguson (1921) has recently stated that it is a parasite of marsupials generally and occurs on rodents and birds Clunies Ross adds the Australian bandicoot* (*Perameles nasuta*) and he states 'the rat is occasionally parasitized by it' But stronger evidence against its being the vector is that it is the common cause of 'tick paralysis,' and if this condition be used as an index of its activities one would expect the distribution of the pseudotyphus and tick paralysis to correspond But it does not The epidemic now reviewed was on a highland plateau whereas tick paralysis is to be found specially all along the coastal region

Moreover, *holocyclus* as its popular name the 'scrub tick' indicates, frequents uncultivated bush land it is not a common tick of well opened up farm lands such as are those on the fertile Darling Downs, the scene of the epidemic which is the subject of this paper nor indeed is it nor any other tick found often on the broader acres of the pastoralist because of his systematic 'dipping' operations It would be more probable that the Mossman fever type or other coastal type is conveyed by the species

2 *Rats and fleas* —Rats and their plague vectors the fleas must be precluded from serious consideration as the distribution of pseudotyphus and plague is not the same At the same time it must be remembered that Strickland (1911) has pointed out that one of the common rat fleas *Ceratophylus fasciatus* is a domestic species while another (*Ctenophthalmus agyrtes*) is a country species Why should not a species like the latter while not being concerned in the epidemiology of plague in towns owing to the special epidemiological circumstances which are connected with it, be responsible for an epidemic of another disease like pseudotyphus in the country? The possibility must be thought of

3 *Rats and mites* —A point in favour of the rat reservoir hypothesis is that in Sumatra recently Walch and Keukenschrijver, during an epidemic of pseudotyphus found that rats while showing no signs of illness had splenic enlargement to the extent of 1 7 the normal size of the organ They also found that 50 per cent of the rats harboured 'mites' which when emulsified and injected into gibbons produced illness On these grounds they concluded that rats are the reservoirs and 'mites' the vectors of the pseudotyphus of Sumatra Fletcher in British Malaya has surmised the same thing

The apparent mouse plague correlation in Queensland was compatible with the rat being the reservoir, for when there is an increase of mice there is also an increase of rats which feed on the former

in

Dr

help in regard to this list A common species of rat mite, viz, *Liponyssus bacoti*, readily attacks man but there is no specific mention of its occurrence in Australia,

* Not the Indian rodent *Nesokia bandicota*

while *Lælaps agilis* has been found parasitic on man there (Cilento, 1923) Dr S Hirst regards species of *Dermanyssus* and *Liponyssus* to be of greater danger to man

4 *Other possible reservoirs and their parasites* —Regarding other possible animal reservoirs Professor Cleland informs me that possibly the fowl tick, *Argas persicus* may be responsible, but that he has never heard of it attacking man in Australia On the other hand *Dermanyssus avium* and *Liponyssus bursa* mites of fowls commonly attack man (Cilento, 1923)

Summary

It will be seen then that there is no particular evidence in favour of any animal other than the mouse being the intermediate reservoir of the virus in Queensland although the rat and its mites which also bite man viz , *Liponyssus bacoti*, *Dermanyssus avium* or *Lælaps agilis* may be concerned There is also no evidence incriminating any particular arthropod as a possible vector

(II) THE POSSIBILITY OF DIRECT TRANSMISSION FROM MAN TO MAN BY THE AGENCY OF A NON DOMESTIC ARTHROPOD

This is discounted by the fact that in Queensland such great distances separate the farms and grazing stations that the general and simultaneous incidence of the cases over a wide area as actually occurred is incompatible with any hypothesis of an arthropod being a direct carrier

Summary of the ætiological evidence regarding the Queensland cases

The mouse seems to be the most likely reservoir and, if it be so the newly discovered *Lælaps australensis* seems to be the most likely carrier

The rat cannot be excluded as a reservoir for it increases greatly coincidently with a mouse plague and a common rat mite, *Liponyssus bacoti*, readily attacks man

There is no evidence that an animal of another order is a reservoir

Ticks seem unlikely vectors largely because of the lack of history of tick bites in the cases and the non correspondence of the epidemic area under discussion with tick paralysis

If ticks be responsible probably the vector is a species of Ixodes which is the only genus common on *Muridæ*

Direct transmission is also contra indicated

THE INDIAN PROBLEM

In the Indian cases, as in the Queensland the dropping nature of their incidence and the fact that a greater number of males is affected, enables us to exclude with some confidence any domestic source of trouble such as lice, bugs, argasid ticks midges and some mosquitoes indeed with regard to lice Megaw (1924) has brought forward many arguments why these insects may be ruled out of further consideration

The alternative possibilities then are, as in the Queensland epidemic, the transmission, (1) by an arthropod vector from an animal reservoir to man, or (2) direct from man to man by an arthropod such as an Ixodid tick, or another mite

(1) An arthropod vector from an animal reservoir

As an animal reservoir is involved in other countries, it seems likely that the same state of affairs exists in India, and the reservoir should be first looked for among the country rodents Failing these other hosts of human ecto parasites must be considered

(a) *The Indian rodents*

A *With ticks* —The list of Indian rodents with their distribution and habits, compiled from Blanfords' Mammalia in the Fauna of British India' and papers in the *Journal of the Bombay Natural History Society* is appended (Appendix II) but before examining it in more detail I will give in view of Megaw s hypothesis regarding a tick being the vector, the following list of the ticks of India that have been found biting man, sent to me kindly by Mr Warburton In this list, as will be seen, no species has a rodent as its *normal* host and the evidence for a tick and a rodent both being involved is therefore slight Further research *vis-a vis* this order may, however, bring to light more evidence in favour of the hypothesis

Ticks found biting man	*Hosts*
Rhipicephalus sanguineus	occasionally on the hare
Hæmaphysalis leachi	occasional hosts *out of* India are —
	Tachyoryctes audax (a mole rat)
	Anomalurus orientalus (a squirrel)
	Acicanthus pumilis (a field rat)
	and in India *Millardia mettada* (a murid)
Hyalomma ægyptium	occasionally on the hare and larvæ and nymphs on the palm squirrel
Ixodes putus	a sea bird species
Larvæ of *Ixodes* and *Amblyomma*	(species not identified)
Ornithodorus savignyi	will feed on rabbits

To this list must be added *Ixodes holoyclus* which, Professor Nuttall informs me has been but rarely obtained in India among its numerous facultative hosts are man, and certain rodents, of which the squirrels seem to be specially favoured *Ixodes acutitarsus* Karsch must also now be added to the list it has recently been collected by Mr Ward at Darjeeling and handed to me by Lieut Col Knowles, I M S, Mr Ward describing it as a very severe biter *

It must be concluded then that if a rodent be the reservoir for the virus in India, and a tick the vector the following should be selected for further investigation

Hæmaphysalis bispinosa with the hare

Rhipicephalus sanguineus with the hare

* A further list of ticks which have been found by us in India to be biting man or in close relation to his person is appended (Appendix III)

Hyalomma ægyptium with the hare or palm squirrel
Hæmaphysalis leachi with *Muridæ*
Ornithodorus savignyi

Evidence indicating the susceptible rodent may be discovered by analysing the relationship between the distribution and habits of the members of this order and the distribution of the Indian cases These seem to occur sporadically all over India but generally as emphasized by Megaw, in a jungly neighbourhood *

The hare hypothesis is compatible with the endemicity of the fever in the Kumaon hills† notwithstanding the fact that hares are reputed not to be addicted to hills *O savignyi* only occurs in South India

The geographical analysis of the Indian rodents and their habits is given in the Appendix see 'Select List,' but it may be said here shortly that the following might be investigated —

The flying squirrels	*Pteromys oral*
The squirrels	*Sciurus indicus* *S palmarum* *S tristriatus* These frequent forests and are often found near villages
The gerbilles	*Gerbillus indicus* which however is not a jungly species it lives on open plains or cultivated fields such as of *jowari* and *bajri*
The long tailed tree mouse	*Vandeleuria oleracea* which inhabits trees palms, bamboos and shrubs and nests in their branches or in the thatch of houses
The rats and mice	*Mus rattus*, *M decumanus* *M musculus* or *bactrianus* *M buduga* *M platythrix* *M mettada* The distribution of all these and their relation to forest cultivation and man is consistent with the incidence of the reported cases of pseudotyphus ‡
Bandicoots	*Nesokia bandicota* and *N nemorivaga* are sometimes found in forests
The Indian bush rat (golandi)	*Golunda ellioti* a jungly species
The porcupines	*Hystrix leucura* of widespread distribution but not jungly
The hares	*Lepus ruficaudatus* of general distribution.

The marmots jerboas, voles hamsters rodent moles mouse hares and certain rare genera need not be considered on the ground of restricted distribution

B *Other mites* —Having now discussed the possibility of tick transmission from a rodent that by other mites must be considered

* For instance in the proximity to the pseudotyphus heavily infected jungly place, Bhimtal, is a Military Camp which is surrounded by cultivated land and is in striking contrast, free from the disease

† It is generally true that the hare is not found in the hills though it has recently been recorded from Nepal and in the Darjeeling submontane region it is quite common up to 4 000 ft

‡ Mr Davidson I C S in an occasional note in the *Journal of the Bombay Natural Historical Society* wrote regarding a plague of rats in the Deccan *The rats seemed to become diseased and died off very fast I think they were troubled by a pale reddish brown tick* (but it is now 40 years ago) Records in the Bombay Secretariat, about 1879—81 would doubtless give much information In the dry hilly villages the rats were almost all Gerbilles elsewhere they were Kol rats and many 'spiny' rats

Mice in Calcutta it was seen above carry a species of *Holostaspis*

The mites of Indian rats are given in Appendix I They include —*Laelaps echidninus* and *L nuttalli* while *Liponyssus bacoti* is a very widely distributed species parasitic on rats although I have not yet obtained it in India *L bacoti* is the only rat mite known to bite man readily The other species of rat *Laelaps* do not attack man *

The possible connection therefore of the disease with rats and *Liponyssus bacoti* must not be forgotten

C *Rat fleas* are not likely transmitters of the condition as in this case rural plague might be expected to show some coincidence with the cases under review

(*b*) *Reservoirs other than rodents*

Animals other than rodents may be the hosts of the vectors of the disease We do not know of any possibility but we will see if any ecto-parasite will indicate one

A INSECTA *Mosquitoes and other midges* the incidence of the Indian cases in rural jungle areas is not incompatible with the jungly species being the vectors but *Ceratopogon Culicoides Phlebotominae* being weak fliers would be more likely to cause house epidemics *Tabanidae* may possibly be implicated

Muscoidea One of the biting or blood sucking muscoids may be a vector

Fleas *Pulex irritans* very seldom finds another host than man cat and dog fleas would be more likely to cause house epidemics than appear to be the rule in these cases Moreover these are very domestic not jungly parasites

Lice the only lice that bite man are special to him and are very domesticated

Bugs would tend to produce house epidemics and are also very specialized and domesticated

One of such species may be a vector but none indicates a natural reservoir of the disease

B ARACHNIDA The connection in other parts of the world of these typhus like fevers with arachnids such as *Dermacentor venestus* and *Trombidium akamushi* has compelled in the analysis above particular attention to the class in spite of the fact that another febrile disorder relapsing fever is related to two very diverse genera *Pediculus* and *Ornithodorus*

Megaw has as has been seen collated a considerable body of evidence that a tick is responsible and has suggested *Rhipicephalus sanguineus* or *Hyalomma aegyptium* A point in favour of the *sanguineus* hypothesis is that it is a comparatively close relation to *Dermacentor venestus* the carrier of Rocky Mountain fever The normal host of *R sanguineus* is the dog but the dog does not act as a reservoir in jail

* There are three

bites man

the Dutch Fa

there are ma

Japanese R

typhus and the presumption is therefore that it does not do so in this jungle typhus The arguments may, however, be fallacious as guinea pigs and some monkeys react differently to jail typhus and Rocky Mountain fever Therefore *R sanguineus* and a non rodent host may indeed be concerned

Transmission by 'mites' from non rodents may be possible, e g, one of the common bird mites of which many species commonly bite man, may be thought of

(2) Direct from man to man by an arthropod

The same rural species which have been above considered might conceivably be the vector of the disease without the intervention of an animal reservoir in which case presumably the culpable one would probably be a very common species and a far traveller But there is of course no evidence in this direction

Summary

While the evidence in favour of a tick being the transmitting agent of Indian pseudotyphus is considerable yet from what is known of the alternative hosts of ticks which bite man one must hesitate to indicate any rodent as a reservoir If any must be it is most likely as Megaw has suggested, the hare or palm squirrel with the vector *Hyalomma ægyptium* or *R sanguineus* Further research into the rodents which live in proximity to man's habitation in conjunction with their parasites is needed

If the association of recorded cases with ticks be only a chance occurrence, which would seem remarkable then rats and *Liponyssus bacoti* or other mites should be enquired into No other dual factors can be suggested

Acknowledgments

In conclusion I must reiterate my thanks to Dr Falkiner for kindly showing me the cases referred to and also state my debt of gratitude to Dr Compston, Director General of Public Health the Commonwealth of Australia, Professors Wood-Jones, Burton Cleland and Harvey-Johnston of Adelaide University and in India Lieut -Col Megaw I M S, Major Sewell, I M S, Director of the Indian Museum and Dr D N Roy for much help in other directions

References

Burton Cleland J (1924) Injuries and diseases in Australia attributable to animals (except insects). *Med Jour Aust* Oct 4

Cilento R W (1923) Random observations on mite infestations of man *Ibid*, Vol I May 19 (20th year)

Ferguson, E W (1924) Deaths from tick paralysis in human beings *Ibid* Oct

Hirst, S (1926) The principal species of Acari parasites on Rats *Jour Ceylon Sci*, D Vol 1 Pt 4

Idem (1926) Descriptions of new mites including four new species of Red Spider *Proc Zoo Soc*, Part 3

Hone, Frank, S (1922) A series of cases closely resembling typhus fever, *Med Jour Aust*, Jan 7th

HONE, FRANK, S (1923) — A further series of cases closely resembling typhus fever *Ibid*, Vol I (10th year), April 21st

Idem (1923) — Further cases resembl ng endemic typhus fever (Brill s d sease) (Supplement to the annual report of the Adelaide Hospital for the year 1923)

Idem (1923) — A problem in Epidemiology *Commonwealth Department of Health* Vol I No 6 June

MEGAW J W D (1917) — A case of fever resembl ng Brill s disease *Ind Med Gaz* Vol LII (No 1 Jan)

Idem (1921) — A typhus like fever in India, possibly transmitted by ticks *Ib d* Vol LVI (No 10 Oct)

Idem (1924) — The typhus group of fevers *Ibid* Feb

Idem with SHETTLE F P and ROY, D N (1925) — Typhus like fever probably tick-typhus in Central India *Ib d* Vol LX (No 2 Feb)

NUTTALL GEORGE H F, (1911) — On symptoms following tick bites in man *Parasitology* Vol IV No 2 July 18

Idem and WARBURTON with OTHERS — Monograph of the Ixodidae etc Camb Univ Press

SEDGWICK, J — *Jour Lom Nat Hist Soc*

STILES and HASSALL — Rats in their relation to public health (No 30) *Public Health and Marine Hosp tal Serv ce Reports* Wash ng ton D C

STRICKLAND, C (1914) — Incidence of plague in Europe *Lancet* Nov 14

WHEATLAND F T (1924) — Some notes on unclassified fevers occurring in the N Queensland Coastal Region *Med Jour Aust* Vol I No 20

Idem (1926) — A fever resembling a mild form of typhus fever *Ibid* (13th year) March 6th

APPENDIX I

MITES FOUND ON RATS

Ladrolaelaps spp Punjab — (Hussain)

Laelaps echidninus Bombay Calcutta — identified by S Hirst

L nuttalli Calcutta — identified by S Hirst

Note —The Laelaps found on rats do not as a rule attack man

L agili s Australian — reported by Cilento (1923) to be parasitic on *M decumanus* and also on man

Liponyssus bacoti — a widely distributed species which bites man readily

Trombicula deliensis Sumatra Walch and Keukenschrijver

T spp (2) novae

T oudemansi ,,

T schuffneri ,,

The last is a common forest mite transmitting pseudotyphus when there are many human cases

Myonyssus decumani
Haemolaelaps sp
Haemogamasus oudemansi
Dermanyssus muris
D sanguineus
— given by S Hirst as occurring all over the world

D gallinae — the common fowl mite frequently found on rats and bites man

APPENDIX II

RODENTIA IN INDIA

		Distribution	Habits
SIMPLICIDENTATA—SCIURAMORPHA			
A SCIURIDÆ (Squirrels and marmots)			
1 *Sciurinæ* (Squirrels)			
1 *Eupetaurus*	*the flying squirrels*	Gilgit 6,000 ft	
a. *cinereus*	woolly		
2 *Pteromys*			
a. *oral*	large brown	Peninsula, Burma, etc	A forest species but lives near villages
b *inornatus*	large red	Mountain Himalayas 6 000—10 000 ft (from Nepal)	As in *oral*
c *magnificus*	Hodgson's	East Himalayas 6—9 000 ft. and Southern Assam hills	As in *oral*
d *yunnanensis*	Anderson's	Southern Assam hills	
e *caniceps*	grey headed	Nepal, Sikkim, etc 4 000—6 000 ft	
f *punctatus*	spotted.	Burma	
3 *Sciuropterus*	*the flying squirrels*		
a. *fimbriatus*	smaller Kashmir	N W Himalayas 6—12 000 ft	
b *alboniger*	particolored	East Himalayas, South Assam hills, Manipur, etc	
c *sagitta*	Horsfield's	Lower Burma.	
d. *spadiceus*	pygmy	Burma	

e *fusc caj llus*	small Travancore	South Indian hills	
f *pearson*	hairy footed	Sikkim etc Assam Manipur etc	
4 *Scu rus*	*the squ rrels*		
a *nd us*	large Indian	General in the peninsula in Manipur	A high tree species of forests rarely coming to the ground
b *b color*	large Malay	East Himalaya Assam Burma etc	Lives in high trees
c *macrurus*	grizzled	South India	a hill forest species
d *f rrugineus*	bay	Upper Burma	
e *locr a*	orange bellied Himalayan	East Himalayas and Assam Burma etc at 8 000 ft	
f *rufigen s*	red cheeked	Burma at 4 000 ft	Dense forest
g *erythræus*	Pallas s	Assam Cachar and Burma etc	
h *qu nque tr atus*	Anderson s	Burma	
i *phayres*	Phayre s	Burma	
j *pygerythrus*	Irrawaddi	Burma	
k *caniceps*	golden backed	Burma	Always with a tree ready for refuge
l *gr se n an s*	grey footed	Upper Burma	
m *locro d s*	hoary bellied	East Himalayas Assam etc East Bengal Burma	
n *atr lorsal s*	black backed	Burma	Bushes near villages rather than high forest
o. *pal naru n*	palm or common striped	General in India including Sind and Baluchistan in more open and cultivated parts especially near human habitation Not in Malabar or East of Bay of Bengal	Not a forest species besides its liking for trees lives in thatch of houses

APPENDIX II—*contd*

		Distribution	Habits
p *tristriatus*	jungle striped	General in India, common in Malabar	A forest species.
q *layardi*	Layard's striped.	Ceylon hills	A forest species.
r *sublineatus*	dusky striped.	South Indian hills	A forest species
s *macclellandi*	striped Himalayan.	Sikkim and Eastern Himalayas Assam hills, Cachar Manipur, Tennasserim	A high forest species.
t. *berdmorei*	Berdmore's.	Burma.	A ground squirrel
II *Arctomyinæ*			
1 *Arctomys*	*the marmots*		
a. *h malayanus*	Thibetan.	Trans Himalayan	
b *hodgsoni*	smaller Himalayan.	Nepal, Sikkim etc	
c. *caudatus*	red or long tailed.	Himalayas north of Kashmir at 8 000 ft	
SIMPLICIDENTATA—MYIOMORPHA			
B DIPODIDAE			
1 *Alactaga*	*the jerboas*		
a. *indica*	the Afghan.	North Baluchistan	Burrows in stony plains
C. MURIDÆ			
I *Platacanthomyinæ.*			
1 *Platacanthomys*	*the spiny mice.*		
a *lasiurus*	the Malabar	Travancore at 2 000 ft	Lives in hollows in trees and damages chillies and jackfruit.

II *Gerbillinæ*			
1 *Gerbillus*	*the gerbilles*		
a *indicus*	the Indian or antelope rat	General in India excepting to East of Bengal	Nocturnal, lives in uncultivated plains and sandy downs often near cultivation In 1878 79 they ravaged the Deccan
b *hurrianæ*	the Indian desert	N W India Sind Punjab, etc at 4 000 ft	Sandy tracts under bushes
c *erythrura*	Afghan	N W India	Lives in holes at the roots of bushes or in sandy banks after near habitations
d *nanus*	little	Baluchistan and Sind	
e *gleadowi*	little heavy footed	Upper Sind and N W India	
III *Murinæ*			
1 *Hapalomys*			
a *longicaudatus*	Berdmore s rat.	Burma	
2 *Vandeleuria*			
a *oleracea*	long tailed tree mouse	General in India except in N W and Burma and Assam ascends to *considerable elevation*	Inhabits palms or bamboo trees and shrubs, nesting in the *branches*, or in roofs of houses
3 *Chiropodomys*			
a *gliroides*	penicillate tailed tree mouse	Khasi hills Burma Manipur	
4 *Mus*	*rats and mice*		
a *rattus*	common Indian	General in India from sea level to 8 000 ft	Burrows in ground and nests in trees Common in houses often nesting in roof A house rat living in thatch
b *concolor*	little Burmese	Burma	Found in all towns and villages along banks of rivers etc, and roads, lives near human habitations

APPENDIX II—*contd*

		Distribution	Habits
c *decumanus*	brown		
d *fulvescens*	chestnut	Nepal and Sikkim	
e *bowersi*	Anderson's	Manipur Karenni Burma	A tree rat
f *blanfordi*	white tailed	Burma Manipur and Khasi hills	
g *berdmorei*	grey	Madras Presidency a hill form	
h *jerdoni*	bicoloured	E Himalayas Khasi hills Tenasserim (a hill species)	
i *niviventer*	white bellied	Himalayas	
j *chiropus*		Kaventri at 4 500 ft	
k *musculus*	common house	India generally except where next species is found	Chiefly in houses sometimes in gardens and fields near villages
l *bactrianus*	Persian house	N W. India and Kashmir	A common house mouse
m *sublimis*	upland	Ladak at 10 000 ft	
n *nitidulus*	Berdmore's	Burma and Sikkim	
o *arianus*	Persian long tailed field	Gilgit at 5 000 ft	In cultivated fields and grassy downs near forests Enters houses in winter
p *buduga*	common Indian field	Peninsula generally not from Indus valley excepting Karachi nor from Himalayas	Burrows in fields gardens woods and sometimes houses
q *cervicolor*	fawn coloured	Nepal E Bengal Assam Khasi hills	
r *platythrix*	brown spiny	Peninsula but not in Bengal	Lives in burrows usually in banks

s. *mettada*	metad or soft furred field	Several parts of the Peninsula, a common rat	In cultivated fields, in any natural hiding place, the rains kill them out.
t *gleadowi*	sand coloured	Sind, Kathiawar, Gwalior	
u *erythrotis*	hairy eared	Khasi hills and Manipur	
v *humei*	Hume's	Manipur	A burrowing genus
6 *Nesocia*			
a *hardwickii*	the short tailed mole rat.	N W India up to 5 000 ft and Purnea (Bengal)	Lives in cultivated and waste land in dry situation
b *bengalensis*	the Indian	Peninsula not on Himalayas without except on common in alluvialia but occurs also on Nilgiris and vale of Kashmere	Lives on cultivated plain gardens and postures in banks of rice fields
c *bandicota*	the bandicoot	Peninsula not in Lower Bengal Sind or Punjab common in Rajputana and South India	Pound cultivated lands and common in villages Also in forest Feeds on grain
d *nemorivaga*	the smaller bandicoot	Purnea Calcutta, East Himalayas Assam Khasi hills Burma	
6 *Acomys*			
a *dimidiatus*	pale spiny mouse	Sind	
7 *Golunda*			
a *ellioti*	Indian bush or gulandi	Very general in Peninsula and Nepal possibly	Lives in the jungle nests in bushes or under them Migratory feeds on dub and other grasses
IV *Cricetinæ*			
1 *Microtus*	*the voles*	Higher Himalayas	
a *roylei*	Royle's	Kashmir, Barendo pass	Burrowing in meadows
b *stoliczkanus*	Stoliczkas	North Ladak	Burrowing in meadows
c *Stracheyi*	Kuman	Kumaon	

APPENDIX II—*contd*

		Distribution	Habits
d *wynnei*	Murree	Murree	
e *blanfordi*	Gilgit	Gilgit	
f *blythi*	Blyth's	Kulu and Himalayas above 13 000 ft	
g *sikkimensis*	Sikkim	Sikkim at 7 000 ft	A forest vole which makes nests of moss
h *melanogaster*	Peri David's	Bhamo	
2 *Ellobius*			
a *fuscicapillus*	Quetta	Near Quetta at 5 500 ft	Mole-like in habits
3 *Cricetus*	*the hamsters*		
a *fulvus*	fulvous grey	Gilgit	Cultivated lands
b *isabellinus*	large grey	Gilgit	Cultivated lands
c *phæus*	little grey	Gilgit	Cultivated lands and pastures and frequently found in houses
V *Spalacidæ*			
1 *Rhizomys*	*the rodent moles or bamboo rats*	Himalayas and Burma	
a *badius*	bay bamboo	Eastern Himalayas and Eastern provinces in hills	Lives in burrows or in high rank grass
b *pruinosus*	hoary bamboo	Assam hills and Burma	Lives in burrows or in high rank grass
c *sumatrensis*	large bamboo		

SIMPLICIDENTATA—HYSTRICOAORA			
HYSTRICIDÆ			
1 *Hystrix*	*porcupines*		
a *leucura*	Indian	Throughout India but not Burma Kashmir and Eastern Himalayan spurs.	Hides among rocks or in caves or burrows Predilection for rocky hills
b *Hodgsoni*	Crestless Himalayan	Himalayas in Nepal and Sikkim up to 5 000 ft and Assam	Hides among rocks or in caves or burrows Predilection for rocky *hills*
c *bengalensis*	Bengal	Lower Bengal, Assam Arrakan *Sikkim*	
2 *Atherura*			
a *macrura*	Asiatic brush tailed	Burma Tippera and Khasi hills	•
DUPLICIDENTATA			
LEPORIDÆ			
1 *Lepus*	*the hares and rabbits*	*Two different species do not usually inhabit the same area*	*Usually live in grass or amongst bushes and rocks.*
a *nigricollis*	black naped	Peninsula South of Godaveri—*Nilgiris*	
b *ruficaudatus*	common Indian	Northern India generally except in W Rajputana, Sind and S W Punjab to the Godaveri—also Deccan and Assam	Lives in waste ground or dry cultivation.
c *dayanus*	Sind.	Sind and *Cutch Indian desert* E of Indus	A desert species
d. *peguensis*	Burmese	Burma but not in Arrakan	Not near the coast or on dense forest.

APPENDIX II—concld

		Distribution	Habits
e *l belanus*	Afghan	Upper Indus valley and Baluchistan at 500 ft	
f *oiostol is*	woolly	Sikkim at high elevations	
g *hyps bius*	upland.	Ladak and Rushku above 14 000 ft	
h *hispidus*	hispid	Himalayan foothills like Terai also Rajmahal hills, Tippera	The sal forest or grass or bamboo land
LAGOMIDÆ			
1 *Lagomys*	*the mouse hares, Pikas or piping hares*	Himalayas	Inhabits burrows among rocks
a *roylei*	Himalaya mouse	Kashmir to Moupin 11—16 000 ft	Lives in rocky ground in pine forests
b *curzoniæ*	Hodgson's	Chumbi valley, Sikkim at great elevations	
c *macrotis*	large eared	Gilgit 7,500 ft and 13 000 ft very locally distributed	Open stony ground
l *rufescens*	Afghan	Bolan pass Quetta District 6 000 ft.	Open stony ground
e *ladacensis*	Stoliczka's	Eastern Ladak Rushku 14 500—19 000 ft.	

SELECT LIST

(Compiled from the foregoing schedule)

Name	Remarks
The flying squirrels *Eupetaurus Pteromys*, and *Sciuropterus* spp	[illegible]
The squirrels (*Sciurus* spp)	The following of the 20 species given in the index have a generalized distribution
	S indicus the large Indian squirrel, a high tree species but rarely coming to the ground.
	S palmarum the palm or common striped squirrel excepting Malabar and the countries east of the Bay of Bengal Not a forest species but likes trees and sometimes lives in thatch of houses It lives on more open and cultivated parts especially near houses
	S tristriatus a forest species common in Malabar the jungle striped squirrel
The marmots (*Arctomys*)	are all exclusively Himalayan
The jerboas (*Alactaga*)	of which there is only one species are exclusively north western
The spiny mice (*Platycanthomys*)	are also restricted to one species inhabiting Travancore only
Gerbillus	has one species the Indian gerbillus or antelope rat, which is generalized in India excepting the countries to the east of Bengal The other species have restricted ranges
	G indicus nocturnal species living in uncultivated plains and sandy downs but often near cultivation In 1878 79 ravaged the Deccan
The long tailed tree mice (*Vandeleuria*)	is a generalized species (excepting Assam and the far north west) It is found at a considerable elevation *V oleracea* inhabits trees palms, bamboos and shrubs nesting in branches or in the roofs of houses
The penicillate tailed tree mice (*Chiropodomys*)	*C glirodes* is distributed in the eastern *countries only*
The rats and mice (*Mus*)	*M rattus* is general up to 8 000 ft and may be further considered, burrows in the ground or nests in trees or roofs of houses which it enters with impunity
	M decumanus is also general though comparatively rare, is found in all towns and villages along banks of rivers and roads and lives near human habitations

SELECT LIST—*contd*

Name	Remarks
	M musculus, the common mouse, is also general excepting the N W and Kashmir where a very close ally, *M bactrianus*, takes its place It chiefly burrows in houses, sometimes in fields near villages and gardens
	M buduga is the common Indian field mouse but is not found in the Indus valley (except Karachi) nor in the Himalayas It burrows in fields gardens and woods and is sometimes found in houses
	The brown spring mouse or leggada
	M platythrix is generalized but not in Bengal Lives in burrows on banks
	The metal or soft furred field rat
	M meltada is nearly general Lives in any natural hiding place in or near cultivated fields The rains kill them out
The bandicoot rats or mole rats (*Nesocia*)	both *bengalensis*, the Indian mole rat, is a *Peninsula* species but not found in the Himalayas, it is excluded from further consideration *Bandicota* and *nemorivaga* seem to be complementary to each other in distribution which would then be general from the Himalayas to Cape Comorin *Bandicota* and *nemorivaga* live near cultivated lands and are common in villages They are also found in forests
The golundas or bush rats (*Golunda*)	are very well distributed in the Peninsula but their occurrence in the hills is doubtful
	G ellioti lives in the jungle nests in or under bushes, is migratory, feeds on dub and other grasses
The voles (*Micr tus* spp)	are all high Himalayan species and not (except *sikimensis*) denizens of the forest
The hamsters (*Cricetus*)	are only found near Gilgit
The rodent moles (*Spalaci dæ*)	have a restricted range in the eastern countries only
The porcupines (*Hystric dæ*)	only *H leucura* has a general distribution except for Burma Pipines are, however not jungle folk
The rabbits and hares (*Leporidæ*)	[illegible] lands or dry cultivation
The mouse hares (*Lagomyid æ*)	are exclusively Himalayan above the 6 000 ft level

APPENDIX III.

LIST OF SPECIES OF TICKS WHICH I HAVE BEEN INFORMED BITE OR WHICH I HAVE FOUND IN INDIA TO BE BITING MAN OR IN CLOSE RELATION TO HIS PERSON

Name	Found by	Place
(1) *Hæmaphysalis bispinosa* larva var *intermedia* is found on the hare *Lepus ruficaudatus* and on *Milardia meltada*	C Strickland	Assam
(2) *Hæmaphysalis aculeata*	Lieut Col McPherson	Bangalore
(3) " sp	Dr Sundar Rao	Chitora
(4) " "	"	"
(5) *Rhipicephalus hæmaphysaloides*	Lieut Col Megaw	Ramgarh, Bhawali
(6) " *sanguineus*	"	Bhimtal
" "	Dr C Strickland	Darjeeling
(7) *Boophilus australis*	Dr Varma	Bhimtal
(8) *Hyalomma ægyptium*	Maj Shettle	Saugor, C P
(9) " " (twice)	Dr Sundar Rao	Chitora
(10) *Amblyomma* sp	Lieut Col Megaw	Calcutta
(11) "	Dr Norrie	Naihati Bengal
(12) "	Lieut Col Megaw	" "
(13) *Ixodes acutitarsus*	M O Mr Ward	Darjeeling
(14) " *holocyclus*		has been rarely (Hussain) found in India (Nuttall), and is a constant parasite of man

Mr Warburton kindly makes the following notes in this connection —

spotted fever

(2) Those normally infesting domestic animals—

Your *Rhipicephalus sanguineus*, *H leachi* and *Hyalomma ægyptium* are sometimes accused of attacking humans without much damage

(3) Those infesting nesting places of birds or lairs of wild animals and attacking people camping in the neighbourhood The best known case is *Ixodes putus* a sea bird tick recorded from the Indian Ocean, but not, I think, In lia

(4) Ticks casually picked up in brush or herbage

These may be *anything*—especially the 'seed ticks" The genera *Ixodes* and *Amblyomma*, having long rostra, are practically ubiquitous, but in most cases of complaint the species was not identified'

DISCUSSION.

Dr U P Basu (Bengal) That a fever very c
does prevail in Calcutta there is not the least doubt
eminent physician Dr Satya Saran Mitra of Howr
in the year 1912 In the August issue *of the India*
I gave a full account of a series of 15 cases, mostly
city There were marked nervous symptoms such
and vomiting seen very early in the course of the
a fortnight the exanthema was hæmorrhagic, ap
and persisted for some length of time As I hac
brought those cases to Col Megaw, whose work ir
well known and whose ready help to the practi
diagnosis of tropical diseases will be gratefully ren
fully examined the rashes and later on showed t
Tropical Medicine who had seen this disease durin
the rashes resembled typhus greatly The possibil
measles having been raised I took Col Megaw wit
my private cases whom I could not bring to his Sc
nervous symptoms and he himself searched for the
rash and oculo nasal catarrh in these cases but d
The question of malignant measles was put out of
in my series and there was no hæmorrhage from th
cases in which Wilson's agglutinin test was done
1 in 30 and the other showed no agglutination wl
diagnosis of typhus owing to lack of biological con
Brill failed to produce the disease in monkeys I

eminent authorities there should be no real difficulty
eruptive fevers such as smallpox measles and scar
character of the eruption Some of my cases were
extracted from three of them Since the publication
many European and Indian medical men practising i
accounts of a very similar disease which they have obs
they *believe to be typhus fever*

It must be admitted, however, that the incidenc
Bengal probably due to the ancient usage of rubbing
hair with mustard oil which keeps away the carriers fro

EXPERIMENTAL YAWS IN PHILIPPINE MONKEYS

BY

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THE results of experiments on yaws in monkeys are briefly presented These experiments extended over a period of three years and followed certain experimental investigations on human volunteers in order to clear up some dark chapters in our knowledge of *Frambœsia tropica* I shall confine myself to a brief summary and conclusions of the work which will appear in full in the March number of the *Philippine Journal of Science*

The main object of these experiments was to find answers to the following questions —

(1) Does *Frambœsia tropica* run the same course in Philippine monkeys as it does in man or can it be induced to do so by special experimentation ?

(2) Does immunity to yaws exist and how does it manifest itself ?

(3) If immunity to yaws exists is it permanent or does it exist only during the stage of infection ?

The answers to these questions are briefly summarized in the following summary and conclusions —

(1) The Philippine monkey is an excellent experimental animal due to its high susceptibility to yaws and on account of the variety of clinical lesions that can be produced experimentally in this animal

(2) The local lesion produced by intra dermal inoculation of Philippine monkeys is a yaw clinically and anatomically identical with that experimentally produced in human volunteers

(3) The early metastatic yaws lesions produced in Philippine monkeys by superinfection—that is to say the typical metastatic yaw, the ringworm yaw, the early frambœsides including psoriasis palmaris—are clinically and anatomically identical with metastatic manifestations of yaws in humans

(4) The late yaws lesions such as the ulcerative form lupus like lesions gangosa, and the late frambœsides such as ichthyotic yaws lesions and the *kerato derma plantare* as produced in monkeys by superinfection are clinically and anatomically identical with these lesions as they occur in man

(5) The duration of incubation of local yaws is the same in Philippine monkeys as it has been established to be in human volunteers

(6) The incubation of the *metastatic generalization of yaws* produced in Philippine monkeys is the same as that found in human volunteers upon experimental inoculation

(7) The duration of early generalized yaws manifestations as well as that of the late ones is much shorter in Philippine monkeys than is *found by* clinical experience to be the *case* in man

(8) However the proportion of the duration of early generalized yaws manifestations to the *duration* of late yaws manifestations *is about* the same in monkeys as in man

(9) The immunity [illegible] resistance [illegible] sions [illegible] set in with Philippine monkeys [illegible] experimentally inoculated human volunteers

(10) The fact that the period of metastatic dissemination of yaws is much *more limited in monkeys than in man is due to the early onset of immunity*

(11) The healing of existing yaws lesions particularly the early *ones* is independent *from* the resistance to superinfection Yaws lesions in the monkey, as in man may heal while the animal *or* the man *is still* susceptible to superinfection and existing lesions will persist a long time after the stage of resistance to new super *inoculation* has fully *developed*

(12) From this it is evident *that* the re inoculability of yaws animals *cannot* be used as a criterion for complete therapeutic sterilization of the yaws infected body organism

(13) The resistance to superinfection once achieved is persistent, *and* no *amount* of treatment can cause the animal once it became resistant, to take infection again

(14) The Wassermann reaction is indefinite and evanescent *in* the case of early *local yaws* Its *strength* and persistence depend upon the duration of *infection* the number of yaws lesions the *intensity* of *the* lesion and to a *lesser* extent on the number of super inoculations

(15) The Wassermann reaction if it became negative due to treatment or spontaneous healing and if all the lesions have *disappeared,* *will reappear* upon unsuccessful superinfection or re inoculation with viable material performed in the resistent *stage*

(16) The serologic reactivity of the *body organism to superinfection* that is the re appearance of the Wassermann reaction and the reactivity *of the organism* to treatment which *manifests* itself as a disappearance of *the* Wassermann *reaction* becomes sluggish *upon* repeated *re-inoculation* and treatment

(17) The re appearance of a positive Wassermann *reaction* can be produced in healed and *cured* animals *without* re occurrence of yaws lesions and, *therefore* a positive Wassermann *reaction* does not *necessarily* mean the persistence of *Treponema pertenue* in the body organism

(18) The focus from which the treponemas are disseminated into the surrounding tissues, or metastatically into remote parts of the body, is the skin

(19) In the lymph glands which correspond to the active lesions *Treponema pertenue* can be found in a fairly high percentage of cases in experimental animals while the early lesion is active, but *Treponema pertenue* was never found in the lymph glands when the lesion had healed either spontaneously or due to treatment

(20) Spontaneous relapses do not occur in experimental monkeys when they reach the stage of resistance The temporary stay of *Treponema pertenue* in the regional lymph gland indicates the route through which generalization in yaws takes place, but it has no significance with regard to possible relapses after a period of latency

(21) The latency in yaws followed by relapse depends upon the time relation between the healing of the existing yaws lesions and the incubation period of the metastatic yaws

AN ATTEMPT TO TRANSMIT *L. ICTEROHÆMORRHAGIÆ* BY *A. ARGENTEUS* AND *A. ALBOPICTUS*

BY

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In the Federated Malay States wide variations in the course of infectious jaundice cause difficulty in arriving at a clinical diagnosis. Fleeting muscular pains, headache, slight catarrh and transient fever may be the only symptoms. It is not surprising that cases are sometimes regarded as mild influenza or dengue fever. Laboratory investigations by Dr. W. Fletcher at the Institute for Medical Research have indicated that such infections are not very uncommon, and the mode of transmission of the virus is therefore a problem of local interest.

Rats in Kuala Lumpur are rarely found to be infected, though numerous carriers exist among the rat population on nearby estates. The causal *leptospira* is known to be capable of penetrating skin and mucous membrane, and the sources of human infection are generally believed to be water, food or mud previously contaminated by the urine of carrier rats.

But the disease is characterized by a leptospiræmia during the early days of its course. Inoculation of guinea pigs with venous blood is usually attended with positive results. Blood cultures in suitable medium are frequently successful and we have even occasionally obtained positive findings from stained blood films on the third, fourth and fifth day of disease. In the tropics and subtropics an abundance of blood sucking insects suggests the possibility of vector transmission.

The subject has already attracted some attention. Noguchi (1918) reported that larvæ and adult *Culex* mosquitoes, larvæ of the house fly and blue bottle, wood ticks and leeches failed to become carriers when fed on infected guinea pigs or on infected organs. Blanc (1920) fed *C. pipiens* on guinea pigs infected with *L. icterohæmorrhagiæ* and inoculated the insects into healthy guinea pigs at intervals of 1, 8, 13 and 30 days after feeding. His results were negative except when the interval was as short as 24 hours. Bonne (1924) unsuccessfully attempted carriage by bed bugs, though he found that the *leptospiræ* survived for two days in the bugs. Some evidence has also been adduced which incriminates a *Tabanid* as a carrier, but the suggestion still awaits experimental proof.

The writer has been unable to find in medical literature any reference to attempts to transmit infection by mosquitoes of the *Aedes* group No doubt can exist that yellow fever is carried by *A argenteus*, and, if the disease does in fact result from infection with leptospiras, it would appear that *A argenteus* might also be an efficient carrier of *L icterohæmorrhagiæ*

Experiments were commenced in this connection early in 1925 and have continued at intervals with both *A argenteus* and *A albopictus* until the present time The distribution of *A argenteus* is patchy in the Federated Malay States, and of recent years the species has practically disappeared from the Kuala Lumpur area The writer is indebted to Dr P S Hunter Municipal Health Officer Singapore for the original supply of adults for the experiment

The Technique Employed

For both breeding and biting a large wooden cage, about 3 feet 6 inches long by 2 feet high, by 2 feet deep was employed The upper 15 inches of the front was covered with mosquito netting Circular holes some eight inches in diameter were cut in the ends of the cage and sleeves of mosquito netting attached to the circumferences A shelf 15 inches above the bottom carried the vessels for breeding

During the breeding out of the first generation healthy guinea pigs were introduced for three hours every second day for feeding purposes Slices of banana were also provided Water was obtained from likely *Aedes* breeding places, filtered through cotton wool and placed in flat trays As the larvæ developed, about half the water in each tray was removed by careful pipetting every second or third day and the quantity made up with freshly filtered water This method gave good results and it was not found necessary to resort to formalinized serum as a pabulum When about 30 adults had emerged the breeding dishes were removed and an infected guinea pig, with numerous *leptospiræ* in the peripheral blood, was placed in the cage The animal was allowed to remain there for 24 hours before removal On the second day, a second infected guinea pig was introduced for a period of 24 hours, after which the floor was mopped with antiseptic solution The female mosquitoes were seen to be engorged with blood

Within an hour or two of the removal of the second infected animal a young healthy guinea pig was introduced and kept in the cage for 12 hours A few females were seen to attack it before its removal Every second day over a period of three weeks, other young healthy guinea-pigs were exposed in the cage for periods of about 12 hours

The procedure was followed once with *A argenteus* and on three occasions with *A albopictus*

Results of the Experiments

Infection of young guinea pigs with *L icterohæmorrhagiæ* is usually fatal The temperature rise is marked and jaundice often occurs In the four experiments described above nearly 50 guinea pigs were exposed to bites from *Aedes* mosquitoes

which had fed on infected animals In no case did jaundice develop and temperatur charts showed practically no abnormality

Very occasionally a guinea pig after infection may have an abortive attack c the disease To determine if any transient infections without appreciable tempera ture reaction had occurred, the animals were bled and agglutination tests carrie out on a culture of the leptospiras It was found that the serum of three guinea pigs which had had abortive infections, caused the *leptospiræ* to lose all motilit within five minutes when examined by the dark ground method With norma guinea pigs serum and also with the serum of those which had been expose l u the mosquito cage, there was no slackening in motility after an interval of hal an hour

At the termination of each series surviving mosquitoes were dissected and film made from the gut and where possible from the salivary glands The number o survivors was only three or four on each occasion, but in no case were *leptospir* seen in the stained films

The writer desires to acknowledge his indebtedness to Dr W Fletcher wh rendered the experiments possible by kindly placing at his disposal the infecte l an immune guinea pigs together with the cultures employed in the agglutination tests

SUMMARY

In cases of Weil s disease the presence of *L icterohæmorrhagiæ* in th peripheral blood during the first week of the disease is usually demonstrable by culture or guinea pig inoculation Positive results from the examination of stained blood films taken on the third, fourth, and fifth day of disease have been obtained Insect carriage therefore seems a possible mode of transmission and from analogy with yellow fever, *A argenteus* would appear to be a possible carrier

A mosquito cage 3 feet 6 inches by 2 feet by 2 feet, was constructed in which some 30 *A argenteus* were bred out Infected guinea pigs with *leptospiræ* in the peripheral blood were introduced for a period of 48 hours The mosquitoes fed well on these animals After removal of the infected animals the floor of the cage was disinfected A young guinea pig was introduced shortly afterwards for a period of 12 hours Other young guinea pigs were placed in the cage for the same period on every other day for three weeks The mosquitoes fed well but none of the animals became infected

Weil s disease has been reported from areas in Malaya where *A albopictus* abounds and *A argenteus* is but rarely seen Accordingly the experiment was repeated with *A albopictus* Although three essays were made none of the young guinea pigs showed signs of infection Three weeks after the original feeding surviving mosquitoes were dissected and examined for *leptospiræ* with negative results

The results of these experiments are not regarded as furnishing conclusive evidence that *Aedes* cannot act as a vector In view of the susceptibility of the

guinea pig to infection, however, it appears improbable that these species function as efficient carriers

REFERENCES

Blanc, G (1920) .. .. *C. R Soc Biol*, Vol LXXXIII, p 263
Bonne, C (1924) .. .. *Idem*, Vol XCI p. 242
Noguchi, H (1918) .. .. *Jour Exp Med*, Vol XXVII, p 609

LE TYPHUS EXANTHÉMATIQUE AU TONKIN.

PAR

BABLET

ET

MESNARD

Une affection rappelant cliniquement le Typhus exanthématique fut signalée dès 1908 en Annam par Yersin et Vassal chez des coolies venant du Tonkin, puis en 1905 à Saigon par Noc et Gautron, enfin au Tonkin en 1921 par H. Coppin. Le diagnostic bactériologique ne put être posé dans aucun des cas signalés et, depuis cette époque, le diagnostic de Typhus semble avoir été écarté au Tonkin.

Au mois de Mars 1926, une enquête bactériologique provoquée par une épidémie fébrile indéterminée sévissant à la Prison Centrale de Hanoi, permit à l'Institut Pasteur de Hanoi récemment créé, d'établir, par les procédés de laboratoire classiques, le diagnostic de Typhus exanthématique.

Les caractères cliniques de la maladie étaient :

Le début brusque avec fièvre élevée, le plateau fébrile à 39-40° à faible rémittance pendant 8 à 10 jours, l'injection des conjonctives et l'angine rouge contrastant avec la pâleur nacrée de la voûte palatine, les symptômes nerveux très accusés, prostration, stupeur ou délire, anorexie complète et constipation, retour brusque à la lucidité coïncidant avec la chute de la température rapide mais en échelons, asthénie tenace pendant la convalescence ou la mort survenue généralement en hypothermie. Aucun malade ne présenta d'exanthème net. Tous les malades étaient des Annamites sur la peau desquels un exanthème discret a pu passer inaperçu.

Mais un gendarme Européen en contact quotidien avec les prisonniers entra à l'Hôpital de Lanessan pour fièvre indéterminée et présenta les signes cliniques du Typhus exanthématique avec un exanthème généralisé des plus nets et réaction de Weil-Félix positive.

L'épidémie se limita aux seuls porteurs de poux (poux de corps et poux de tête). L'épidémie fut incapable de se diffuser à l'extérieur de la prison, sauf toutefois le gendarme signalé précédemment, un coolie de l'Hôpital indigène et deux miliciens fréquemment en contact avec les prisonniers malades. Ils présentèrent une affection cliniquement semblable au Typhus exanthématique

avec réaction de Weil Félix positive ce qui fait supposer qu'ils furent contaminés par eux

Des mesures ayant été prises pour épouiller les prisonniers, l'épidémie s'arrêta immédiatement

Le nombre des malades évacués de la Prison Centrale sur l'Hôpital indigène du 20 Novembre 1925 à fin Mars 1926 pour fièvre indéterminée rappelant cliniquement le Typhus exanthématique s'élève à 150 et la lecture du registre d'infirmerie permet de supposer que 150 cas légers ont évolué à l'intérieur de la prison 16 décès se sont produit parmi ceux évacués sur l'hôpital la mortalité s'élèverait donc à 5, 3 pour cent environ Ces chiffres sont approximatifs quoique étayés par les séro diagnostics rétrospectifs pratiqués en Mars sur les prisonniers dont la maladie remontait à Janvier ou Février *

La suite de l'enquête nous a permis de constater que le Typhus exanthématique existait à l'état endémique au Tonkin Depuis le mois d'Avril 1926 jusqu'à fin Août 1927 nous avons pu dépister 110 cas sporadiques repartis dans les principales villes du Tonkin du delta et de la région frontière 96 chez les Annamites, 14 chez les Européens Sauf 5 enfants tous les malades étaient des adultes exerçant les professions les plus diverses tant à la ville qu'à la campagne

Le tableau clinique du Typhus sporadique ne présente guère de différence avec celui du Typhus épidémique constaté à la prison de Hanoi Les symptomes nerveux sont souvent moins accusés et la convalescence est plus courte Les Européens adultes à l'exception d'un seul, présenterent tous de l'exanthème Les Annamites eurent 5 fois un exanthème net †

La mortalité fut très faible 2 cas chez les Annamites 1 cas chez les Européens

Il est plausible d'admettre pour ces cas que le pou est encore l'agent de transmission Au Tonkin les porteurs de poux sont en effet nombreux et par conséquent susceptibles d'être contaminés dès l'enfance et au cours de l'existance ce qui expliquerait la bénignité de ces cas sporadiques et le manque apparent de contagiosité

Nous avons eu recours dans les recherches de laboratoire à deux méthodes classiques l'inoculation expérimentale et le séro diagnostic de Weil Félix

Nous avons d'abord éliminé par les hémocultures et les examens de sang à l'état frais ou après la coloration d'autres affections fébriles possibles Au cours de l'épidémie de la prison les inoculations de sang de malades au cobaye nous ont donné 7 résultats positifs sur 9 Elles furent faites suivant la technique de Ch Nicolle L'ascension thermique indice de l'infection de l'animal a généralement débuté entre le 9e et le 12e jour après l'injection et le virus a été conservé au laboratoire de Mars 1926 à Juin 1927 par 42 passages sur cobayes

Pour les cas sporadiques, 3 inoculations au cobaye pratiquées dans des mauvaises conditions ont été négatives

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* Notre enquête a été facilitée à la Prison de Hanoi par les observations de M le Professeur [illegible] chargé du Service Médical de l'établissement pénitencier

† La plupart des observations de cas de Typhus sporadiques constatés dans la région de Hanoi sont dues à l'obligeance de M le Dr Malfre M[illegible] de l'Assistance chargé du Lazaret

La technique employée pour le séro diagnostic de Weil Felix fut l agglutination *macroscopique* totale observee a la loupe apres une heure d etuve a 37° ou apres 8 heures a la temperature de laboratoire

Les souches employees furent

Au cours de l épidémie de 1926 les souches Proteus X19 Syrie et Metz provenant de l Institut Pasteur de Paris

52 reactions nous ont donne 27 résultats positifs

Depuis Decembre 1926 nous avons ajoute aux souches precedentes les souches 67 et la souche Kinsbury dues a l obligeance de MM Fletcher et Lesslar (Institute for Medical Research Kuala Lumpur)

Les souches Metz Syrie et 67 sont indologenes la souche Kinsbury anindologene

Durant cette époque sur 42 serums provenant de malades atteints de *Typhus* sporadique nous avons observe que 5 sérums agglutinaient exclusivement la souche anindologene Kinsbury 23 agglutinaient exclusivement les souches indologenes et 11 simultanement les souches indologenes et anindologenes

Nous n avons tenu compte que des cas ou le taux d agglutination etait egal ou superieur à 1 100

Nos observations nous permettent de conclure que le Typhus exanthematique existe au Tonkin sous la forme sporadique durant toute l annee et se manifeste sous la forme epidemique lorsque sont realisees les conditions de promiscuite et d'hygiene defectueuse

THE DIAGNOSIS OF YELLOW FEVER

BY

W H HOFFMANN, M D,
Professor Laboratorio Finlay, Cuba

LAST year the writer had the opportunity to prove the endemicity of yellow fever in West Central Africa from the histological examination of a number of cases So the yellow fever problem is of great practical importance for the countries round the Indian Ocean

In this case, after all the negative results of ten years' work the histological method of diagnosis was superior to all the others The lesions in the liver are so extraordinary that they always allow of a diagnosis which may be supported by the lime casts in the kidney

In slight endemic cases the clinical diagnosis may remain impossible Also the first epidemic cases generally were overlooked, though in a murderous epidemic the cases do not leave much doubt The clinical diagnosis depends on the albuminuria and the incongruence between pulse and temperature but always a very careful observation of the course of the disease is necessary because all the other symptoms are very inconstant and varying

The bacteriological diagnosis is without practical value on account of its difficulty only exceptionally it has been possible to find the *Leptospira icteroides*

The diagnosis from the specific anti bodies of the serum does not help in the first days of the disease, but it is useful to form a retrospective opinion on cases that have passed the disease

Yellow fever diagnosis is not easy still if an epidemic is developing it should always be possible The public health authorities of the Far East may easily come into the situation that they have to make the diagnosis of yellow fever Only if the first case is immediately detected, it is possible to avoid disastrous epidemics

NOTE SUR LA PATHOGÉNIE DE LA DENGUE

PAR

HENRY G. S. MORIN,

Institut Pasteur de Saïgon

CHANDLER ET RICE au cours de la grande épidémie de Dengue au Texas en 1923 ont réussi à transmettre la maladie dans quatre cas sur six avec des Stegomyia argentea gorgés 24, 48, 72 et 96 heures auparavant sur des patients (Poiret) atteints depuis un à cinq jours.

D'autre part Siler, Hall et Hitchens en 1925 à Manille ont échoué dans toutes les tentatives de transmission avec des moustiques infectés depuis moins de 11 jours. Dans trois cas, au contraire, les piqûres, inactives jusqu'au 10e jour, ont transmis la maladie à partir du 11e jour et jusqu'à la mort de l'insecte.

Cette divergence n'a pas passé inaperçue. Siler, Hall et Hitchens la signalent sans se l'expliquer. Chandler émet l'hypothèse de la transmission héréditaire possible du virus par l'insecte à ses œufs. Cependant il garde, avec plusieurs auteurs, l'impression que les faits épidémiologiques s'accordent mieux avec un pouvoir infectant assez précoce chez l'insecte.

Il n'est donc peut être pas sans intérêt de verser au débat la relation d'une petite épidémie de dengue éclatant dans une collectivité si bien circonscrite et dont les circonstances ont permis une surveillance si serrée, que l'on a pu saisir avec précision le début exact et l'enchaînement le plus vraisemblable des cas à partir du premier rigoureusement isolé (Poiret).

L'aviso Craonne (700 tonnes, 125 hommes d'équipage) arrive à Saïgon fin Février 1926. Le médecin du bâtiment fait campagne à ce bord depuis un an et forcément, dans un milieu aussi restreint, connaît individuellement chacun de ses hommes. Malgré un travail assez dur en pleine saison chaude, nécessité par des travaux de réfection du navire, l'état sanitaire se maintient excellent. Seul un matelot, qui avait obtenu trois jours auparavant une permission pour se rendre au Cap-Saint-Jacques, présente brusquement le 28 Avril une fièvre très élevée avec rachialgie violente. Il est hospitalisé. Une enquête très précise établit que tous les officiers, tous les matelots du bord sont en parfait état de santé.

D'ailleurs le seul malade observé est en voie de guérison, le 4 Mai il est apyrétique et rétrospectivement l'ensemble des phénomènes pathologiques, la courbe thermique, le rash, le résultat négatif de toutes les investigations du laboratoire imposeraient le diagnostic de dengue si ce cas n'était pas totalement isolé. Il n'existe

pas de poussée épidémique à cette époque dans la ville de Saigon et d'autre part il est impossible malgré une nouvelle enquête dirigée cette fois spécialement en ce sens de dépister dans le personnel du bord un état pathologique qui ressemble même de loin à un cas fruste de dengue

Pendant tout leur séjour à bord les matelots ont couché sur le pont du navire la coque métallique surchauffée pendant le jour rendant pénible le séjour la nuit dans les entreponts Les stégomyias pullulent dans la partie de la rivière où l'aviso est stationné

Le 8 Mai le navire entre au bassin de radoub l'équipage est débarqué et couche à la caserne Francis Garnier dans des meilleures conditions de confort (moustiquaires)

Le 15 Mai 3 matelots présentent en même temps un état pathologique ayant les mêmes caractéristiques que la maladie du matelot atteint le 28 Avril et à partir de cette date au rythme de un à trois par jour de nouveaux cas se produisent de telle sorte qu'en moins de quarante cinq jours 80 pour cent de l'effectif Européen a payé son tribut à l'épidémie Les services ont été désorganisés Bref le diagnostic épidémiologique est évident, sans parler des constatations cliniques multiples qui concordent également de façon parfaite comme le montre le dépouillement de 150 observations de malades des formations maritimes voisines auxquelles l'épidémie s'est évidemment étendue Ultérieurement se produit à Saigon une poussée épidémique dont il est plus difficile préciser la marche en raison sans doute d'un certain degré d'immunité acquise de beaucoup de résidants (Hoiret)

En résumé le premier malade infecté au Cap Saint Jacques tombe malade le 28 Avril Il est le point de départ d'un foyer épidémique caractéristique qui se constitue à partir du 15 Mai à bord du Craonne Ce foyer rayonne sur les formations maritimes voisines et donne un grand nombre de cas typiques

Les faits épidémiologiques ici ont donc paru cadrer exactement avec les faits expérimentaux établis par Siler Hall et Hitchens La vérification expérimentale de l'hypothèque de Chandler serait donc des plus intéressantes à tenter car si elle ne pouvait être faite il deviendrait peut être possible de considérer que la dengue observée à Manille et à Saigon est une affection distincte de la maladie observée par Chandler et Rice au Texas ou que l'incubation du virus chez l'insecte peut varier sous l'influence des conditions du milieu extérieur

PROTOZOOLOGY.

ON THE INFLUENCE OF THE THYROID GLAND ON THE COURSE OF A PROTOZOAL INFECTION

BY

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Professor of Protozoology,

AND

B M DAS GUPTA,
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Calcutta School of Tropical Medicine

OUR ignorance with regard to the factors which underlie resistance or susceptibility to protozoal diseases is at present profound Thus, we do not know why *Entamœba histolytica* in one person causes amœbic dysentery, whereas in nine persons infected out of ten it only causes the carrier condition, which is almost free from symptoms Children in endemic malarial areas tend to become 'salted,' so to speak, with chronic malarial infection, an infection which at first tends to cause high fever, later a low grade of fever, still later, an afebrile tolerance to infestation with the parasite If we could find out something of the mechanism of natural susceptibility or resistance to protozoal infections, our methods of treatment and of prophylaxis with regard to such infections might be much improved

It was suggested to us by Lieut Col H W Acton, I M S, that we should undertake an investigation into the possible rôle of the endocrine system with regard to such susceptibility or resistance, commencing with the thyroid gland as the great regulator of the body mechanism

In doing so, it was first necessary to select a suitable protozoal parasite and suitable laboratory animals for study Infection with *Entamœba histolytica* is transmissible chiefly to kittens, and even then only with some degree of uncertainty, the question of the pathogenicity or otherwise of the intestinal flagellate protozoa is still unsettled, whilst malaria is transmissible only to the higher apes, and even in them causes only a transient infection (Mesnil and Roubaud, 1917, 1920) On the other hand, trypanosomiasis is an infection which particularly lends itself to

laboratory study, and in which mathematical and statistical observations can be carried out The most readily available pathogenic trypanosome of animals in India is *Trypanosoma evansi*—the parasite of surra, and accordingly we decided to work with this parasite The strain used by us was one which was very kindly supplied by Mr W Taylor F.R.C.V.S Principal Punjab Veterinary College, Lahore, it was isolated in the first instance from a horse suffering from surra then inoculated in sequence into each of two dogs and finally passaged into rabbits which were sent from Lahore to Calcutta The laboratory animals which we used were rabbits monkeys (*Macacus rhesus*) guinea pigs and—chiefly—white rats

Before proceeding to give an account of our findings it may be as well to contrast the course of infection with a non pathogenic and with a pathogenic trypanosome respectively in experimental animals This subject has been admirably dealt with in the memoirs by Taliaferro and Taliaferro (1922) and Taliaferro (1923) and is summed up in a final memoir by Taliaferro (1926)

Taking infection with *T lewisi* in the rat as a typical example of infection with a non pathogenic trypanosome the course of the infection is recorded by Taliaferro and Taliaferro (1922) as follows (Fig 1) After an incubation period of four days trypanosomes first appeared in the rat's blood At this period of first invasion of the blood stream the trypanosomes show the most extraordinary diversity of shape size and form whilst the coefficient of variation of length was at the very high figure of 25 32 per cent The number of trypanosomes present now multiplied very rapidly until at the 10th day after inoculation there were 338 000 trypanosomes present per c mm Meantime however the curve of increase of the total number present rose by more and more gradual increments whilst the trypanosomes present became much more monomorphic and tended to assume an adult type By the 10th day the coefficient of variation was at a figure of only 3 95 per cent This is due to the production in the blood plasma of substances which inhibit the reproduction of trypanosomes but which do not prevent those trypanosomes present from growing up to the 'adult' type

From the 10th to the 14th day the number of trypanosomes present dropped from 338 000 per c mm to 76 000 per c mm the drop being attributable either to phagocytosis of trypanosomes—a phenomenon first examined by Laveran *and* Mesnil (1901) or to trypanolysis From the 14th until the 35th day the infection remained at a very low level the total infection on the 35th day being 45 000 trypanosomes per c mm of blood Finally the few trypanosomes remaining in the blood stream suddenly disappeared their disappearance being *attributed* by these authors to the production of trypanolysins in the blood The rat is now immune to the infection and cannot be re infected *again with T lewisi*

In the case of the pathogenic trypanosomes, matters are entirely *different* Here the infection tends to be of one of two different types In the first, the *infection* is a hyper acute one, the animal shows no trace of resistance at all, and dies

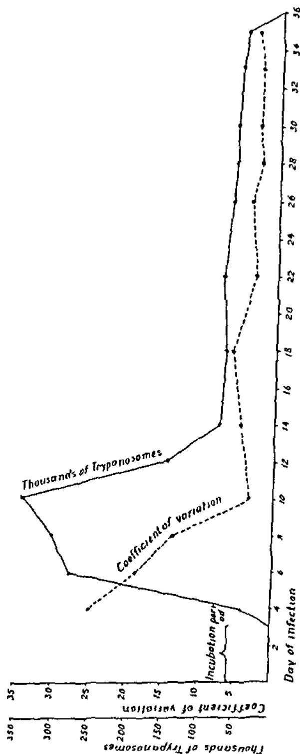

Thousands of Trypanosomes
Coefficient of variation
Incubation period
Day of infection
Coefficient of variation
Thousands of Trypanosomes
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
5
10
15
20
25
30
35
50
100
150
200
250
300
350

Fig 1

at the height of the infection This is shown in Fig 2, from Taliaferro and Taliaferro (1922) In the second as shown in Fig 3, from Taliaferro and Taliaferro (1922), the infection tends to assume a chronic or relapsing type and the animal dies after a more or less prolonged period of increasing anæmia and emaciation it may even die at a time when no trypanosomes can be found in its peripheral blood

This work of Taliaferro and Taliaferro has been of special interest to us on account of the general similarity of our findings with *T evansi* with their general findings for other trypanosomes pathogenic to laboratory animals

After trying several different methods for counting trypanosomes we finally adopted the standard technique advocated by Kolmer (1915) In this a drop of the fresh blood is first examined in order to judge roughly the degree of infection present and to judge roughly what degree of dilution of the blood will be necessary The blood is then diluted either in the leucocyte or in the erythrocyte pipette of a hæmocytometer apparatus with a special diluting fluid consisting of

Formalin (40 per cent)	2 c cs
Glacial acetic acid	2 c cs
Distilled water	96 c cs

to which 2 c cs of Ziehl Neelson s carbol fuchsine is added After the dilution the blood must be very thoroughly shaken in the pipette in order to avoid agglomeration of the trypanosomes The counting chamber of the hæmocytometer is next filled ten minutes are allowed for the trypanosomes to settle to the bottom of the chamber and the total number of trypanosomes present over the entire square ruled area is then counted In doing this we have found that artificial light is far preferable to daylight

In measuring trypanosomes it is necessary to adopt a uniform and standard method After trial of different methods we adopted the following uniform technique Thin blood films are taken from the infected animal before they dry they are exposed to the vapour of 4 per cent osmic acid for 30 seconds fixation is then completed by allowing methyl alcohol to act on them for five minutes The films are then stained for 20 minutes by diluted Giemsa s stain and allowed to dry in air They are then mounted in a projection drawing apparatus and the outlines of 100 consecutive unselected trypanosomes are drawn The stage micrometer scale is then placed in the same apparatus and drawn on the same sheet of paper A pair of dividers is next set to one micron on this scale a pencilled line is drawn freehand down the middle of the length of each trypanosome and its total length including that of the free terminal portion of the flagellum, is stepped off in microns with the dividers In this way the coefficient of variation of length of the trypanosomes present can be calculated This coefficient will be high when rapid multiplication of the trypanosomes is occurring and low when multiplication is ceasing or has ceased

In passaging a standard dose varying from 10 000 to 5 000 000 trypanosomes, was invariably given intraperitoneally The total number of trypanosomes present

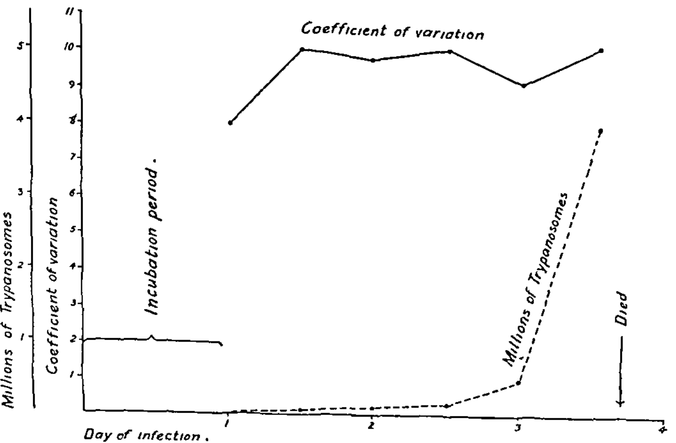

Coefficient of variation
Incubation period.
Millions of Trypanosomes
Died
Millions of Trypanosomes
Coefficient of variation
Day of infection.

Fig. 2.

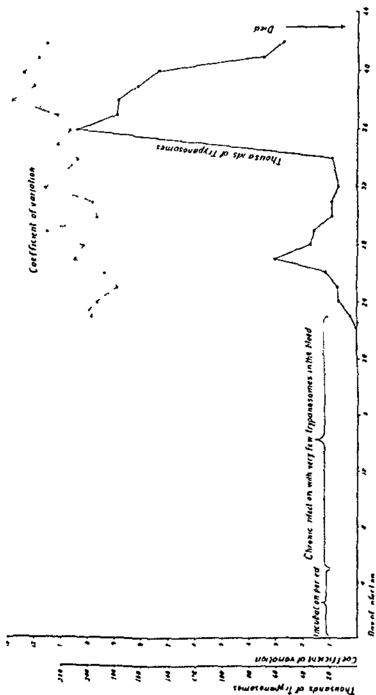

Died
Thousands of Trypanosomes
Coefficient of variation
Chronic infection with very few trypanosomes in the blood
Incubation period
Thousands of Trypanosomes
Coefficient of variation

Fig 3

in the blood was first counted, the blood was then diluted to the degree requisite and the intraperitoneal injection given with a tuberculin syringe

In common with many previous workers we have found that the course of surra infection varies widely with the different species of animal experimented with In guinea pigs (nine animals observed) the infection tends to be of chronic or relapsing type death occurring some 10 to 90 days after injection Two animals out of nine recovered spontaneously, and were in good health 139 days after injection In rabbits (15 animals observed) the disease is also of the chronic or relapsing type with intervals when trypanosomes are absent from thin films of the peripheral blood During these negative intervals however, trypanosomes can usually be demonstrated in the blood by taking a sufficient quantity of it

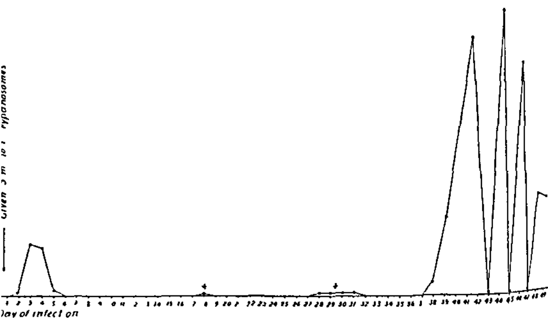


Fig 4

hæmolysing and centrifuging Trypanosomes are often to be detected in thick blood films, where examination of thin blood films has been with negative results We have completely failed to find any evidence of any latent or intracellular phase of *Trypanosoma evansi*, though films and sections from the viscera of several animals at the negative phase were searched for such phases

Figure 4 shows the typical course of surra infection in the rabbit In general it will be seen that this graph closely resembles Fig 3 from Taliaferro and Taliaferro During the course of chronic surra infection in the rabbit emaciation often becomes very severe, although the animals are on a liberal diet whilst blepharitis, keratitis and conjunctivitis were especially noticed It so happened

that on one day nine infected rabl its all failed to show trypanosomes in thin blood films one quarter of a c c of blood was taken from each hæmolysed in ? c c of acetic tartaric acid solution in small test tubes centrifuged and films

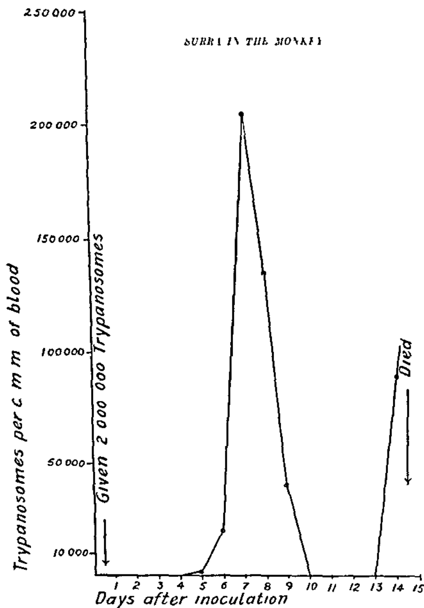


Fig 5.

in the blood was first counted the blood was then diluted to the degree requisite and the intraperitoneal injection given with a tuberculin syringe

In common with many previous workers we have found that the course of surra infection varies widely with the different species of animal experimented with In guinea pigs (nine animals observed) the infection tends to be of chronic or relapsing type death occurring some 40 to 90 days after injection Two animals out of nine recovered spontaneously and were in good health 139 days after injection In rabbits (15 animals observed) the disease is also of the chronic or relapsing type with intervals when trypanosomes are absent from thin films of the peripheral blood During these negative intervals however trypanosomes can usually be demonstrated in the blood by taking a sufficient quantity of it

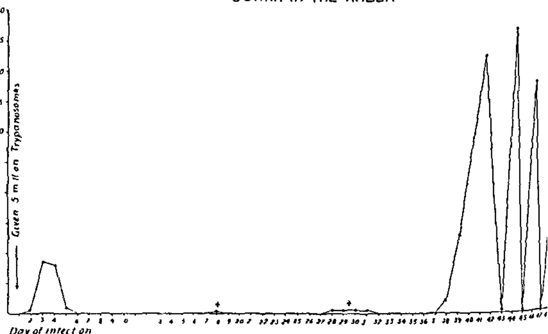


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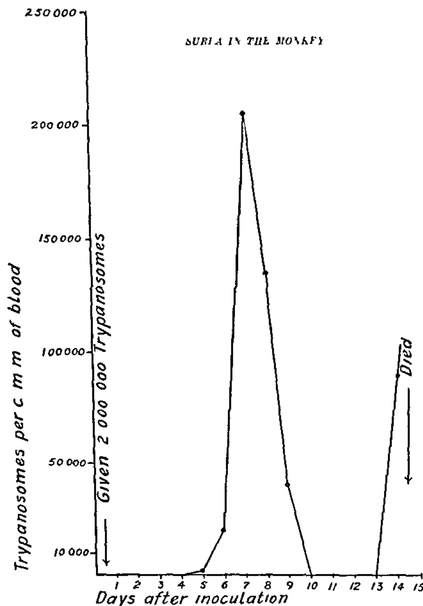


Fig 5a

prepared and stained from the deposit In seven of *these preparations trypan*osomes were found by this technique A remarkable feature of surra in the rab is that often—indeed usually—death occurs when the total trypanosome count *falling or when trypanosomes have* been absent from the peripheral blood for more or less prolonged period At post mortem examination of these anim either lobar or broncho pneumonia is almost invariably present, and it wou appear that the surra infection lowers the general resistance of the animal to su an extent that death is more often due to secondary complications than to t primary trypanosome infection

Monkeys (*Macacus rhesus*) are much less resistant to infection with surra th are guinea pigs and rabbits and in these animals surra tends to be an acu disease with a rapidly rising trypanosome count and death within a period of few days Of ten normal monkeys inoculated however, one showed a low gra infection for a fortnight and then recovered spontaneously, it was alive an in good health with no trypanosomes present in thin or thick blood films, t months after injection The incubation period in the monkey is about five day and death tends to occur about the 14th to the 15th day Lobar pneumonia *an almost invariable terminal complication in the monkey Fig 5 shows th* typical course of surra infection in *Macacus rhesus*

Most of our observations however, were on white rats, of which 88 norm animals were inoculated (two from infected rabbits 18 from infected guinea pig and 68 from other infected rats) In the first series of rats used, the virus w maintained in guinea pigs and rats inoculated with guinea pig blood—a standar dose of 1 000 000 trypanosomes being given The mean incubation period to fir appearance of trypanosomes was 1 8 days and death occurred in from 4½ to 8 (mean 6 4) days By constant sub passage from rat to rat however, the viru became so exalted in virulence that death usually occurred within 60 hours o inoculation of the same dose The disease in the white rat is of hyper acute type the count rising with very great rapidity, and the rat showing not a particle of evi dence of any resistance at all This is illustrated in Figs 6 and 7, which show th typical course of surra in the white rat There is no evidence in such curves of th production of any substance in the blood plasma which inhibits the reproduction *of the trypanosomes Just prior to the death of the rat the trypanosome count* which was previously rising with great rapidity may suddenly and rapidly fall *owing* to trypanolysis and apparently conditions in the dying host are unsuitable for the trypanosomes

To some extent there is a partial—but only partial—correlation between the *dose of trypanosomes injected* and the interval to death, a small dose taking longer to kill than does a big one During the acute phase the lung appears to be the organ most heavily involved, whilst the bone marrow of infected rats appears *to be curiously free from trypanosomes* The final and rapid fall in the trypanosome count appears to be brought about by trypanolysis In the course of this process the flagellum together with the parabasal body, is thrown bodily

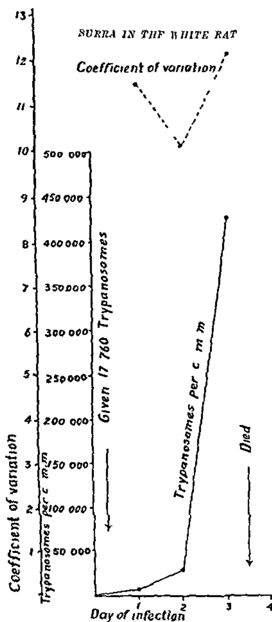

SURRA IN THE WHITE RAT
Coefficient of variation
Coefficient of variation
Trypanosomes per c mm
500 000
450 000
400 000
350 000
300 000
250 000
200 000
150 000
100 000
50 000
Given 17 760 Trypanosomes
Trypanosomes per c m m
Died
Day of infection

Fig. 6.

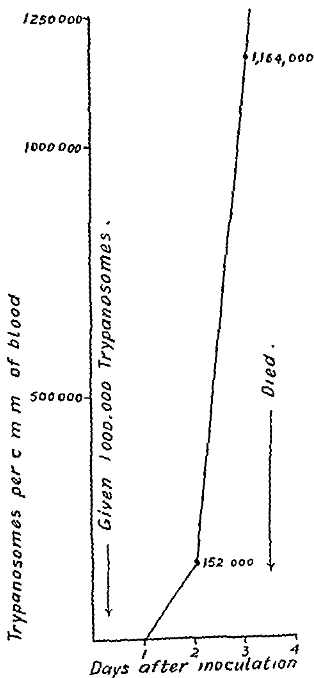

SUPPA IN THE WHITE RAT
1250000
1000000
500000
1,164,000
152 000
Trypanosomes per c m m of blood
Given 1000.000 Trypanosomes.
Died.
1
2
3
4
Days after inoculation

Fig 7

out of the cell The nucleus (trophonucleus) breaks down by karyorrhexis, and as it does so the posterior half of the trypanosome becomes filled with rounded fragments of chromatin In the cytoplasm of the polymorphonuclear leucocytes numerous granules of chromatin are seen, these appear to be nuclear remnants of trypanosomes which have been ingested The spleen appears to be the chief site of trypanolysis So rapid is this destruction of trypanosomes that films

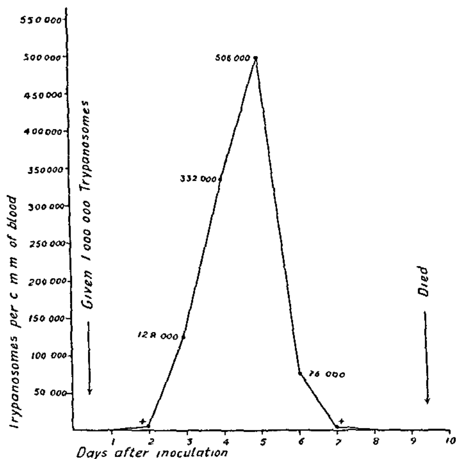


Fig 8

taken from the blood and viscera half an hour after the death of a rat whose blood may have been swarming with trypanosomes half an hour before its death may fail to show a single trypanosome

In a very occasional rat however, there is some evidence of resistance and the course of the infection tends to assume a more relapsing and chronic course This is well exemplified by Fig 8, from Rat 115 where after the normal rise of the

count the count fell to nil and no trypanosomes were found in the blood on the two days preceding death In these animals as the count is rapidly dropping a very peculiar phenomenon occurs which we may term the 'bone-marrow reaction' There is a sudden invasion of the blood by large numbers of erythrocytes of an extraordinary type They are larger than the normal erythrocytes usually about one and a half times the diameter of the normal erythrocytes They stain a deep purplish colour with Giemsa s stain and look as if the membrane of the erythrocyte was unusually tough Many of them give the impression that they still retain the lens body of the immature erythrocyte They do not hæmolyse in the aceto formalin solution used for the trypanosome count These big tough deeply basophilic erythrocytes appear to be immature erythrocytes from the bone marrow and the fact that normoblasts also appear in association with them suggests that their appearance in the peripheral blood is the result of a sudden reaction in the bone marrow

Our observations show that *Trypanosoma evansi* is a very monomorphic trypanosome The mean length of 6 400 trypanosomes measured in blood films from healthy rats (including the free terminal portion of the flagellum) was $23\ 60 \pm 2\ 833\mu$ with a mean coefficient of variation in length of 12 0 per cent

In order to observe the effect of loss of thyroid secretion on the course of the infection monkeys (*Macacus rhesus*) were taken and a sub total thyroidectomy carried out one quarter of each lobe of the gland being left on each side in order to just conserve the life of the animal For the carrying out of these operations we are very much indebted to Lieut Col H W Acton I M S An interval of ten days was then allowed to elapse in order that any excess of thyroxin present in the tissues might be burnt up before inoculation The control normal monkeys were boxed at the same time as the thyroidectomized ones and all animals were kept under identical conditions of housing feeding etc in order as far as possible to eliminate any other factors which might influence the course of the disease In all 11 thyroidectomized and 11 normal control monkeys were used The normal controls were inoculated at the same time and with the same dose of virus from the same source as in the case of the thyroidectomized animals and the course of the disease studied by the methods already indicated

The result of previous thyroidectomy in the monkey we found was to markedly increase the severity of the disease in the animal and to shorten the time interval to death Thus the thyroidectomized animals died in an average of 9 5 days after inoculation as against a mean of 14 5 days for the normal controls In brief with the loss of thyroid secretion the resistance of the animals is markedly lowered and none of the chronic type of infection with relapses which may be seen among the controls occurred Further instead of death being associated with lobar ... to be

1

The typical course of the infection in the thyroidectomized monkey is shown in Fig 9 (from thyroidectomized monkey No II)

In order to study the effects of intensive thyroid feeding white rats were taken, weighed and caged under identical conditions of housing and feeding To half of them a daily feed of 1 mg in the first experiment and 2 mg in the second of desiccated thyroid extract (P D & Co 's and B W & Co s) was given for ten days

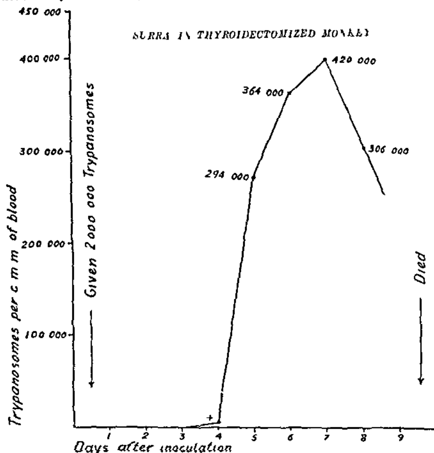


Fig 9

In giving the feeds the desiccated tablet was weighed emulsified in milk with gum acacia and a measured dose corresponding to either 1 mg or 2 mg administered by dropping it into the mouth with a capillary pipette So far from the rats disliking the feeds they lapped them down readily and appeared to enjoy them

The first result of this intensive thyroid feeding was as unexpected as it was bizarre Thyroid gland substance is frequently given to patients with a view to

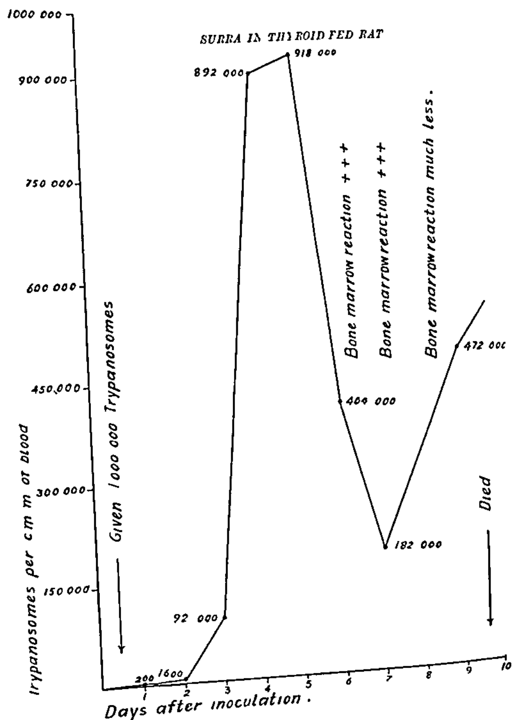

SURRA IN THYROID FED RAT
1000 000
900 000
750 000
600 000
450,000
300 000
150 000
Trypanosomes per c m m of blood
Given 1000 000 Trypanosomes
200
1600
92 000
892 000
918 000
404 000
182 000
472 000
Bone marrow reaction +++
Bone marrow reaction +++
Bone marrow reaction much less.
Died
1
2
3
4
5
6
7
8
9
10
Days after inoculation.

Fig 10

reduce their weight It is usual for normal white rats when taken from the pen caged individually and put on to a liberal diet to put on weight and most of the control rats in these experiments did so But the thyroid fed rats put on weight at an excessive rate Rat 50 put on 33 grms in ten days or more than 30 per cent of its original weight Rat 68 which started with a weight of 141 grms, reached a weight of 183 grms on the tenth day and in size resembled a guinea pig rather than a rat A dose of 1 mg of thyroid extract to a rat weighing 100 grms corresponds roughly to a corresponding dose of about 10 grains to a man of 60 kilos weight It would seem that with intensive thyroid feeding the basal metabolism is very markedly stimulated and in a small cage with but little exercise possible and an unlimited diet the paradoxical result is obtained that the weight goes up instead of down

In all thyroid feeds were administered to 21 rats with 15 normal controls On the 10th day both thyroid fed and normal control rats were injected at the same time with the same dose of trypanosomes from the same donor The thyroid feeds were thereafter continued daily until the death of the animal

The results with thyroid feeding were much less consistent than those after thyroidectomy Taken in groups there is not very much appreciable difference between the incubation period and time interval to death as between the thyroid fed rats and the normal controls But here and there an individual thyroid fed rat put up an amazing resistance and the disease tended to change from its usual hyper acute type in the normal animal to a more chronic and relapsing type in the thyroid fed rat This increased resistance is well shown in Fig 10 from thyroid fed rat No 30 which survived for as long as nine days after a dose of 1 000 000 trypanosomes Together with this increased resistance the bone marrow reaction previously described became very prominent indeed in fact the reaction is far better studied in thyroid fed rats than in normal controls In all 3 out of the 21 thyroid fed rats showed a very marked increase in resistance when compared with the 15 normal controls

Both after thyroidectomy and after thyroid feeding the differences which appear were much more marked with certain *individual* animals than with groups of animals and any correlationship that may exist between the state of thyroid activity and the susceptibility or resistance of the animal to the disease is of only a partial character

This may be explained by the work of Schern (1925) Working with *T brucei* *T equiperdum* and *T rhodesiense* this worker has shown that death in acute trypanosomiasis is associated with a condition of acute hypoglycæmia The trypanosomes appear to live directly upon the blood sugar, and to use it up At first the liver responds by an increased output of glycogen Finally however the strain upon its glycogen metabolism becomes excessive, a condition of absolute hypoglycæmia sets in and the animal dies

In order to test whether this occurs with *T evansi*, we carried out a final experiment details of which are shown in the following Table

TABLE

	Sex	Weight grms		TRYPANOSOMES PER C MM OF BLOOD				Killed on	Blood-sugar percentage
				1st day	2nd day	3rd day	4th day		
Rat 125	F	93	Each given 20 000 trypanosomes from Rat 124	272 000	340 000	78 000		3rd day	0·100
Rat 126	M	93		0	1 100	102 000	330 000	4th day	Nil
Rat 127	F	90		0	0	9 400	316 000	4th day	Nil
Rat 128	M	7[illegible]		0	200	48 400	298,000	4th day	Nil
Rat 129	F	98		0	0	57 000		3rd day	Nil
Rat 130	F	83		0	+	73 000	2[illegible]6 000	4th day	Nil
Rat 131	M	1[illegible]2	Normal controls						0·085
Rat 132	F	88							0·071
Rat 133	M	93							0·086

* + denotes that trypanosomes were present in the blood but in numbers too scanty to count

Nine rats were weighed and caged on the same day, under identical conditions of housing and feeding To each of the first six a dose of 20 000 trypanosomes from the blood of rat No 124 was given intraperitoneally These were killed either on the third or the fourth day, when the infection was at its height, and their blood sugar immediately tested At the conclusion of the experiment, the three non inoculated normal controls were also killed and their blood sugar titrated For these estimations we are very much indebted to Dr J P Bose, Diabetes Research Scholar, Calcutta School of Tropical Medicine It will be seen that in only one out of the six trypanosome infected rats was the blood sugar normal and it is to be noted that in this rat the total trypanosome count was rapidly falling In the other five rats there was not sufficient blood sugar present to give a positive test

We may conclude, in general, that trypanosomes live on the blood sugar This explains why the control of the thyroid gland over the infection is only a partial one The blood sugar content is governed by the activity of the liver and of the adrenal glands, the control of the thyroid gland over the liver and adrenal glands is but a partial one Hence the only partial correlation between the degree of thyroid activity and the course of the disease We hope at some future date, if time and circumstances permit, to resume this enquiry, and to study next the rôle of the adrenal glands in connection with infection with *Trypanosoma evansi*

REFERENCES

KOLMER, J A (1915) — A method of transmitting known numbers of trypanosomes, with a note on the numeric relation of trypanosomes to infection *Jour Inf Dis* Vol XVII p 79

LAVERAN, A and MESNIL F (1901) — Récherches morphologiques et expérimentales sur le trypanosome des rats (*Trypanosoma lewisi* Kent) *Ann Inst Pasteur*, Vol XV, p 7673

MESNIL F and ROUBAUD E (1917) — Sur la sensibilité du chimpanzé au paludisme humain *C R Acad Sci* Vol CLXV, p 39

Idem (1920) — Essais d inoculation du paludisme au chimpanzé *Ann Inst Pasteur* Vol XXXIV p 466

SCHERN K (1925) — Ueber Trypanosomen I—VI Mitteilungen *Centralbl f Bakt* Abt Orig Vol XCVI pp 356 360 362, 440 444 451

TALIAFERRO W H (1923) — A study of size and variability throughout the course of pure line infections with *Trypanosoma lewisi*, *Jour Exp Zool* Vol XXXVII p 127

Idem (1926) — Host resistance and types of infection in trypanosomiasis and malaria *Quart Rev Biol* Vol I, p 246

Idem with TALIAFERRO, L G (1922) — The resistance of different hosts to experimental trypanosome infections with especial reference to a new method of measuring this resistance *Amer Jour Hyg*, Vol II, p 264

DISCUSSION

Dr R H H Goheen (Bombay) In the case of death associated with autolysis of the trypanosome and without the factor of other intercurrent disease such as pneumonia is it probable that protein toxin may be the cause of death ?

Col S L Brug (Netherlands East Indies) I think Col Knowles is to be complimented on his paper There is a mystery in medicine and that is why so many people infected with pathogenous germs escape disease Now this mystery has not been solved by Col Knowles paper, but a tip of the veil covering it has been lifted and a way for further research has been indicated

Lieut Col R Knowles I M S (Bengal) In reply to Dr Goheen death in trypanosomiasis appears to be of two types (*a*) There is death primarily due to acute trypanosomiasis This possibly is due to protein shock from the products of trypanolysis but it is always associated with absolute hypoglycæmia In such cases the administration of glucose might be tried (*b*) In chronic trypanosomiasis death appears to be more often due to a secondary infection such as pneumonia Here the trypanosome infection knocks out the resistance of the animal causing increasing anæmia and emaciation It thus becomes more susceptible to secondary infections which may kill it Death may occur days or weeks after trypanosomes have disappeared from the blood stream

PRELIMINARY OBSERVATIONS ON THE MORPHOLOGY AND LIFE HISTORY OF *SPIROCHÆTA ANSERINA*

BY

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THERE are many gaps in our knowledge of the exact morphology and life history of the spirochætes. For instance, such questions require to be answered as:—What is the exact morphological structure of a spirochæte? Does it possess flagella? Or, if not, then to what is its motility due? In the case of hereditary transmission of spirochætal infections, what is the exact mechanism of this transmission? Have spirochætes an intracellular phase in either vertebrate or invertebrate host? Is there or not such a thing as a 'granule phase' in the life history of spirochætes? Finally, there is still considerable confusion with regard to both the systematic position and classification of the spirochætes.

In trying to answer these questions many workers have worked with *Spirochæta anserina*, the parasite of avian spirochætosis, since this spirochæte is readily obtainable, and the strain can be easily maintained in the laboratory. Thus Balfour (1907) described an 'after phase' of the infection in birds from whose blood the spirochætes had disappeared; this was associated with the appearance in the nucleated red blood corpuscles of deeply staining rounded intra-corpuscular bodies which he thought might be latent forms of the spirochætes, and which might grow up into spirochætes. Hindle (1912), however, considers that these granules of Balfour are the result of karyorrhexis of the nucleus of the red cell, and other workers agree with him. Franchini (1924), for instance, records a similar appearance in the blood of birds not infected with spirochætosis, and Gerlach (1925) failed to find them in Austria, where fowl spirochætosis occurs.

Several workers have shown that the disease is transmitted by ticks of the genera *Argas* and *Ornithodorus* though chiefly by *Argas persicus* and the development of the spirochæte in *Argas persicus* has been extensively studied The infection will pass into the second tick generation and as demonstrated by Hindle (1912) the second generation without re infection may hand on the infection to the third generation Hindle (1912) has given an account with a diagram of the life cycle of *S anserina* in the fowl and in the tick which has now been adopted as a standard account and which has found its way universally into the text books He describes the formation of coccoid' bodies from spirochætes in the blood of the bird whilst in the tick the spirochætes are supposed to invade the cells of the body especially of the Malpighian tubules and in these as also in the lumen of the gut they break down into granules which multiply till very large numbers are produced These granules are supposed to be inoculated into the fowl or to invade the tick ova and to grow up again into spirochætes If the ticks be kept at 28°C this granule phase is especially well seen, whilst if ticks in *this condition be transferred to the warm incubator at 37°C the granules become* converted into spirochætes Hindle s observations it is to be noted were made on stained films only On the other hand Marchoux and Couvy (1913) entirely deny the existence of the granule phase in the tick they consider that the granules in the cells of the Malpighian tubules are a normal structure of the tick and have found them in several different types of insect in the absence of spirochætal infection They state that when a tick has once taken in the spirochætes these are constantly present in the body fluid though they are often so fine and delicate in starving ticks that special staining methods have to be employed for their demonstration

The spirochætes enter the salivary glands and when they cannot be found in other tissues of the tick they are still present in the salivary ducts When the eggs are laid by the tick they are coated with fluid from special secretory glands this fluid contains spirochætes which penetrate the eggs even passing through their chitinous envelopes and as many as thirty spirochætes may be present in a single ovum

In May 1927 the Indian Research Fund Association very kindly sanctioned a grant for the commencement of a Spirochætosis Inquiry at the School with a view to try and solve once and for all some of the questions asked at the commencement of this paper and as *Spirochæta anserina* was the most readily available spirochæte for laboratory study we have worked with this organism In the present paper we desire to present a brief account of our findings to date in this enquiry

We expected that it would be very easy to obtain infected birds and for this purpose searched the blood of many fowls and some ducks from the Tiretta bazaar in *Calcutta Curiously enough we* have been absolutely unable to demonstrate the existence of fowl spirochætosis in Calcutta *Argas persicus* abounds in the fowl runs and the birds in the Calcutta bazaars must come from many different sources up country but we have never found an infected fowl or tick in Calcutta The exact geographical and seasonal distribution of the disease in India has still to

be worked out though Reaney (1907) has shown that it occurs in Central India and Montgomery (1908) that it is especially prevalent in the north of the Punjab and in the North West Frontier Province during the cold weather It is also known to occur during the rainy season in Poona As we were unable to obtain either infected ticks or infected fowls in Calcutta Mr J T Edwards Director Imperial Institute for Veterinary Research Muktesar very kindly supplied us with a strain and work was commenced in May, 1927

Cycle in the Vertebrate Host

In the vertebrate host we have studied the life cycle by the aid of stained blood films and smears sections of the viscera and above all by direct observation under the dark ground It is impossible to over emphasize the value of the dark ground apparatus in such work Over and over again when we have failed to detect spirochætes in blood films or in smears of organs we have found them in fresh material under the dark ground whilst in working on the tick cycle we have come to rely upon the dark ground microscope almost to the exclusion of all other methods

In all 126 fowls were inoculated in each case half a c c of infected blood being inoculated into the wing vein Out of these 7 failed to show the infection at any time and remained in good health Our first finding was that fowl spirochætosis (as seen in Calcutta at least) is not a relapsing fever at all we have failed to obtain a single relapse with any of the birds injected The incubation period after the injection of half a c c of infected blood is almost invariably 24 hours though in an occasional bird spirochætes do not appear in the blood until 48 hours after injection The bird then has a single attack of fever the temperature remaining elevated by about 1° C for 2 to 7 days and either dies during the acute attack or very shortly after it or recovers At first the strain proved very virulent and killed the majority of the birds since then however it has become increasingly less virulent and at present the greater majority of our fowls recover Rapid emaciation is a feature of the disease towards the termination of the disease when it proves fatal the bird s head droops and finally there is a curious and persistent backward retraction of the head the bird lying paralysed with its head markedly retracted and its eyes closed Death may occur as early as 24 hours after inoculation or as late as 27 days after the mean observed being 6 45 days

Once spirochætes appear in the blood they multiply exceedingly rapidly in numbers but usually persist in the blood for only one or two days We have seen the infection clear within 24 hours of the first appearance of spirochætes or to persist for as long as 7 days the mean of these observations being 2 5 days As the crisis approaches the spirochætes gather into enormous tangles each containing dozens of spirochætes These tangles grow ever larger and larger and their appearance—both in the vertebrate and in the invertebrate host—appears to be always prior to death and disintegration of the spirochætes Once the tangles have formed the tangled mass of spirochætes soon becomes immobile and the

individual spirochætes break down into granules and disintegrate We have been absolutely unable to confirm the supposed phase of formation of coccoid bodies in the blood, the tiny granules which are formed are, we believe, the result of death and disintegration of the spirochætes

Division of the spirochætes is invariably by binary, transverse and never longitudinal fission Towards the height of the attack certain curious very long 'jointed' forms appear *in scanty numbers These appear to consist of a single* very long spirochæte which is about to divide, not into two, but into 3, 4, 5, or even more individuals, these often lying at an angle to one another, so that the entire form shows joints and open angles

During the phase when the peripheral blood is positive, spirochætes are found in emulsions of all the internal viscera Thus in birds killed at the height of the attack we have found spirochætes in the kidneys, lungs, brain, testes, ova, bone-marrow and spleen We have come across no intracellular forms, either in stained smears or in sections of the viscera, and we believe that no such phase occurs Once the crisis is over, we have been unable to find spirochætes in any of the internal viscera except the brain In the brain occasional scanty spirochætes may be found for 2 to 3 days after the crisis, and this would appear to be correlated with the marked central nervous system symptoms, the paralysis and head retraction, etc

We have seen the after phase described by Balfour, but believe that it has nothing to do with fowl spirochætosis What happens in infected birds which recover from the attack is that they either become quite healthy and fit, or else become sick and anæmic and die off after a more or less prolonged interval of days or weeks Our control non inoculated fowls, however, showed the same thing, and we ascribe these deaths to crowding of the fowls in cages, and much handling of them by sweepers and laboratory attendants Fowls bought direct from the Calcutta bazaar have been kept under very unhealthy conditions as a rule, and the natural mortality rate amongst them is high Possibly the conditions in the Khartoum bazaar from which Dr Andrew Balfour got his fowls were the same During this period we have seen the granules in the erythrocytes referred to by Balfour in 6 out of 22 fowls examined, but always in extremely scanty numbers, some 4 or 5 for instance in a film They are also to be seen in blood films from the internal viscera occasionally We have seen all phases from the giving off of a bud of chromatin from the nucleus to the relatively large rounded mass of chromatin lying free in the cytoplasm of the erythrocyte, whilst in some films the process appeared to have gone further, *and a very few extra cellular granules were observed* We believe that these granules are the result of karyorrhexis of the erythrocyte nuclei, poisoned by the toxins of the disease

We have carried out immediate post mortem observations on many birds which have died in this so called after phase, also in control non inoculated birds which died under similar conditions of housing The chief changes found were marked karyorrhexis and karyolysis in the endothelial tissue generally especially

in the lungs Blood culture of the heart blood has invariably remained sterile No spirochætes or forms which could be interpreted as any latent or intracellular phase of the spirochæte were observed In fact the birds appear to die of general inanition in spite of a liberal diet

In searching films made from the internal viscera during the height of the attack and after the crisis we have looked especially for any evidence of phagocytosis of spirochætes by the leucocytes or by endothelial cells but have found none The destruction of the spirochætes at the crisis appears to be entirely brought about by the production of lysins in the blood plasma

Cycle in the Invertebrate Host

(*a*) *At room temperature* Having dissected many *Argas persicus* captured in the Tiretta fowl bazaar in Calcutta without finding any of them infected with *Spirochæta anserina* we used ticks from this source for our observations on the cycle in the invertebrate host

Fifty eight ticks were fed on birds at the height of the infection by the method advocated by Patton and Cragg (1913 Plate LXXXII) the fowl having its head swathed in a muslin cap the ticks being inserted into the cage which is surrounded by butter muslin and the ticks being allowed to engorge themselves These were subsequently kept at room temperature between 82 5° F and 98 8° F and dissected at different intervals after the feed A few observations on stained films from the different viscera convinced us that this method of examination is nothing like as successful as direct examination of emulsions of the viscera under the dark ground and up to the present time our observations on the cycle in the invertebrate host have been carried out entirely by examination under the dark ground microscope

Of the 58 fed ticks 7 failed—for some reason or another—to become infected The other 51 all took They were dissected at different intervals of time after the infective feed and emulsions of the contents of the anterior diverticula posterior diverticula mid gut rectal diverticula brain coxal gland testis or ovary white gland uterus and cœlomic fluid examined under the dark ground We found spirochætes in the following organs —

Intestine or diverticula in	40
Salivary glands in	25
Coxal gland in	7 out of 35 female ticks
Malpighian tubules in	4
Cœlomic fluid in	21
Testis in	2 out of 16 male ticks
White gland in	6 out of 16 male ticks
Ovary in	3 out of 35 female ticks
Uterus in	2 out of 35 female ticks
Brain in	12

Summarizing our observations, we may state that we believe the life cycle of the spirochæte in *Argas persicus* to be as follows —

Of the ingested spirochætes some 85 to 90 per cent die off They accumulate in the gut and in the diverticula in ever increasing tangles, become immobile, and disintegrate Under the dark ground these *tangles of disintegrating spirochætes* look like woolly, fleecy, silvery clouds, and they may be so large that a single tangle may occupy the entire field of the microscope The remaining 10 to 15 per cent however survive These are of two types, the vast majority are normal and very actively motile spirochætes, many of which are in process of binary transverse fission A few exceedingly long 'jointed' forms are seen, however These are spirochætes which are about to divide into 3 4, 5, or even more young forms, and show open angles between the dividing individuals

By incessant division of the motile spirochætes, the gut gradually comes to contain abundant spirochætes of very small, thin, and fine type The change in the morphology of the spirochæte, as this occurs, is very remarkable The ultimate product of this incessant multiplication is the production of a *type of spirochæte* only about one third or less of the length of a normal spirochæte, of extreme thinness, but exceedingly active with regard to motility We may perhaps refer to these forms as 'tenue' forms By degrees the gut comes to contain more and more tenue forms, and these accumulate especially in the anterior diverticula The Malpighian tubules are not invaded to any extent (positive in only 4 out of 51 infected ticks), and when they are infected, it would appear that only occasional scanty spirochætes from the rectum get into the tubules Infection of the Malpighian tubules does *not* appear to be an essential part of the developmental cycle in the tick

From about the 6th day onwards, these delicate 'tenue' forms invade the cœlomic cavity of the tick, and from it, come to infect all the viscera of the tick Thereafter the residual forms in the gut slowly die off, and no motile spirochætes are observable in the contents of the gut and diverticula, as a rule, after the 18th day, though occasionally a very few motile individuals may be found up to the 31st day *after the infective feed*

As ordinarily observed under the dark ground, *S anserina* does not appear to possess terminal flagella, the reason being that in such fluids as blood or the intestinal contents of fed ticks the field is full of myriads of brightly lit points of light and the general diffusion of the light prevents the terminal flagella from being seen As seen under the dark ground in the cœlomic fluid of fed tick, however, *S anserina* is seen to possess an undoubted very delicate single terminal flagellum at each end Here the cellular content of the cœlomic fluid is very scanty, one gets a jet black background, and the very thin, delicate terminal flagellum at each end of the spirochæte is well seen It is about one fourth to one fifth of the length of the spirochæte, and often projects at an angle to the spirochæte

From the cœlomic cavity these spirochætes invade all the different viscera of the tick, and they are to be found in the brain especially, also in the coxal gland,

ovary, and uterus of the female tick and in the testis and white gland of the male tick A curious point which we have noticed is that, whereas the spirochætes are actively motile in other organs they are usually dead when found in the white gland in the male tick it is possible that the white gland contains some inhibitory substance

Although they invade all the viscera of the fed tick however the spirochætes tend especially to accumulate in the salivary glands which were found infected in 25 out of 51 positive ticks (i e ticks which showed spirochætes in the gut or other tissue) In the salivary glands the 'tenue' forms rapidly develop into spirochætes of normal length and thickness dividing forms are very frequently seen and although the infection in the glands is never a very heavy one yet it is progressive As the cycle in the gut dies out, that in the salivary glands appears to increase and develop The earliest period at which the salivary glands are invaded appears to be the sixth day after the infective feed, and we have found that fed ticks are infective via the bite to clean fowls on the seventh day after the feed Our observations on this point so far however are but few and it is possible that the tick is infective at a date earlier than the seventh day after the feed We have seen motile spirochætes in the salivary glands up to the 31st day after the infective feed

The transmission cycle in *Argas persicus* thus appears to be a very simple one and in no ticks so far have we seen any evidence of a granule phase or of any special involvement of the Malphighian tubules The cycle consists of incessant division of the surviving spirochætes in the gut with—finally—the production of 'tenue' forms which are of extreme delicacy and very actively motile these invade all the viscera of the tick, but especially the salivary glands (which lie in close apposition to the anterior diverticula) Infection is normally transmitted via the saliva and the bite, but occasionally the secretion of the coxal glands is also infective

(*b*) *In the cool room* Thirty three ticks which had fed on fowls at the height of the infection were kept in the cool room, temperature between 60° F and 85° F and were dissected at different intervals after the feed The cycle appeared to develop in these ticks in much the same manner as in ticks kept at room temperature but whereas 12 per cent of ticks kept at room temperature failed to become infective only 6 per cent of those kept in the cool room failed to become infective and it would appear that the infection takes better at lower than at higher temperatures an observation which may perhaps be correlated with Montgomery's observation that in the Punjab fowl spirochætosis is especially liable to become epidemic during the cold weather

Spirochætes were found in these fed ticks as follows —

In the gut or diverticula in	30
Salivary glands in	12
Coxal glands in	1
Cœlomic fluid in	10

Malpighian tubules in	2
Testes in	2 out of 9 male ticks
White gland in	1 out of 9 male ticks
Ovary in	2 out of 24 female ticks
Uterus in	1 out of 24 female ticks
Brain in	3

The earliest period at which motile spirochætes were observed in the salivary glands in this series of ticks was at the 6th day

* * * * * * *

We have not so far especially studied the mechanism of hereditary transmission *in the tick but it is to be noted that motile spirochætes were present in the ovaries* of 3 out of 66 fed female ticks examined a proportion of 3 6 per cent and it appears to us likely that the ova come to be infected *in situ* in the tick prior to their fertiliza tion and oviposition With regard to salivary gland infection the percentage of positive ticks (counting from the 6th day after the infective feed) which showed motile spirochætes in the salivary glands was 62 5 per cent for ticks kept at room temperature and 54 5 per cent for those kept in the cool room There appears to be but little difference between the infectivity with regard to the two sexes of 26 male ticks dissected 2 (or 8 per cent) were negative of 66 female ticks dissected 7 (or 10 6 per cent) were negative but these figures are probably within the range of random sampling for the total numbers observed

In conclusion we would like to emphasize that this paper is of a preliminary character only and that our work and observations are still in progress

REFERENCES

BALFOUR A (1907) A peculiar blood condition probably parasitic in Sudanese fowls *Jour Trop Med and Hyg* Vol X p 153

Idem. (1908) Spirochætosis of Sudanese fowls *Third Report Wellcome Research Labs Khartoum* p 38.

Idem (1911) The role of the infective granule in certain protozoal infections as illustrated by the spirochætosis of Sudanese fowls *Jour Trop Med and Hyg* Vol XIV p 113

Idem (1914) Notes on the life cycle of the Sudan fowl spirochæte *Trans XVII Inter Cong Med* London Sect 21 Part 2 p 275

FRANCHINI G (1924) *Observations sur les hematozoa res des oiseaux d Italie* *Ann Inst Pasteur* Vol. XXXVIII p 470

GERLACH F (1925) Geflu ssp rochatose in Oesterreich *Centralbl Bakt* I Abt XCIV p 45

HINDLE E (1911) On the life cycle of *Sp rochæta gallinarum* Preliminary note *Parasitology* Vol IV p 463

Idem (1912) The inheritance of spirochætal infection in *Argas persicus* *Proc Camb Phil Soc* Vol XVI p 457

MARCHOUX, E and COUVY, L (1913) .. Argas et spirochætes. Premier memoire. Les granules de Leishman. Deuxieme partie Le virus chez 18 acariens. *Ann Inst Pasteur*, Vol XXVII, 450, 620

MONTGOMERY, R E (1908) .. .. On a spirochæte occurring in the blood of chickens in India. *Jour Trop Vet. Sci III*, Part I, p 1.

PATTON, W S and CRAGG, F W. (1913) .. 'A Textbook of Medical Entomology', Madras

REANEY, M F (1907) .. Spirillosis of domestic fowls *Brit Med Jour*, Vol I, p 1118 *Ind Med Gaz*, Vol XLII, p 401

DISCUSSION

Dr P A Dalal (Bombay) . The finding by Col Knowles of the jointed long forms reminds me of a similar form I saw from the blood of a guinea pig inoculated with the spirochæte of rat bite fever It would be interesting to know if this appearance is at all common in the fowl, as such forms are extremely rare in case of the rat bite spirochæte

Major R B Lloyd I M S (Bengal) Asked if Col Knowles would give further information as to the disease, not from the laboratory standpoint, but especially as to its epidemiological features

Lieut Col R Knowles, I M S (Bengal) in reply to Dr Dalal The long jointed forms are rare in the fowl, but relatively common in the fed tick They are very long germs consisting of one individual dividing into 3, 4, 5 or more individuals set at open angles to one another

In reply to Major Lloyd I am afraid that the subject has hardly been investigated as yet in India We have Montgomery's report that fowl spirochætosis is epidemic in the Punjab and N W F P. [illegible] ther It is also known to occur in the Central I[illegible] I have been trying to get into touch with [illegible] to the veterinarians to collect the information with regard to the geographical and seasonal incidence of the disease in India, and to let us have it.

sans flagelles, que j'ai appelé la *tête*, le moyen constitué par une organelle en forme de *Sablier* qui est le *centroblepharoplaste* et qui d'un coté s'articule avec la tête, y faisant hernie, et de l'autre coté avec le troisieme segment ou le corps proprement dit Le polymorphisme du parasite est dû à plusieurs facteurs d'abord, à sa partie mobile qui est le second segment, tous les mouvements se passant autour de l'articulation inferieure du sablier et se faisant en avant, en arriere et latéralement, la *tête* les suivant et donnant souvent, lorsque l'animal est vu de face, des figures plus ou moins rondes ou l'on distingue 4 cercles concentriques le premier correspondant à l'extremite antérieure du sablier, le second a la circonference de la tête hyaline, le troisieme a la périphérie d'une organelle qui entoure le centroblepharoplaste et que j'appele la *campanula* et le quatrième au pourtour du corps, ce dernier cercle etant le seul qui puisse être moins regulier que les autres Le second facteur du polymorphisme est l'active contractilité de la partie antérieure du *corps* correspondant a la zone des fibrilles divergentes qui ont la fonction de vrais *myonemmes* *Le troisieme facteur, enfin, est la contractilité sarcodique du corps* du protozoaire

Outre ces variations subies par le même individu, il y a d'autres provenantes de leur configuration *ex initio* et dont la finalité nous est inconnue Souvent le corps nous montre un appendice uni par un si mince segment qu'il semble que bientot nous allons assister à une sorte de bourgeonnement Et cependant ni telle separation se produit, ni la structure de tels parasites nous en donne une hypothese explicative

L'etude des preparations colorees nous habilite a bien apprendre la cytologie du protozoaire Elle comprend

I —*Premier segment ou tete* Celle ci se laisse tres rarement colorer par l'Hemalun de Mayer ou l'Hematoxyline à fer d'Heidenhain Seules, deux fibrilles situées a la base et representant probablement sa limite inférieure, se montrent sous *forme de deux barbelles siderophyles s'insérant aux angles du pôle supérieur du sablier*

II —*Second segment* Très complexe, il comprend deux organelles —

(*a*) l'une interne, tubulaire, en forme de *sablier* dont l'étranglement, *in vivo*, se donnerait à l'union de son tiers supérieur et moyen et que j'ai appelé le *cou* Très siderophyle, souvent uniformement coloree, elle perd dans ces preparations colorées sa forme en sablier pour devenir une sorte de faisceau pyramidal, montrant quelquefois un retrécissement vers le tiers anterieur Dans les prépara tions bien differentiées on voit que ses bords sont épais, siderophyles et la lumiere depourvue de toute substance Il n'est pas rare de rencontrer sur sa longueur une bande transversale siderophyle située au niveau *du retrecissement Son* pôle superieur possede une *calotte hemispherique* surmontant une *ménisque transversale superieure* qui, quelquefois, déborde la largeur du sablier La *calott hémispherique peut* prendre divers aspects. Elle est cotoyée par deux expan sions que je nomme *labelles* (Planche XVI, figs 2, 4) La *menisque* transversale superieure, qui peut avoir une structure granuleuse, donne insertion à l'organelle

denommée *campanula* dont il s'agira a suivre Le pôle inferieur du centroblépharoplaste est plus large s articule avec le *corps* du parasite et est pourvu d innombrables granules basaux qui dans les preparations bien différentiées s alignent en deux bandes paralleles formant la *double menisque transversale inferieure* organelle assez importante comme nous le verrons plus loin

La tige en sablier est entourée d une fine ligne d ectoplasme qui se continue avec le corps

(*b*) une formation infundibiliforme en cone tronque entoure le centroblépharoplaste s insérant par son vertex sur la menisque transversale supérieure (Planche XVI fig 3) et la base bien plus large descend vers le corps a une hauteur variable mais n excedant pas beaucoup les limites inférieures des *myonemmes en eventail* situés dans le 5eme ou 6eme superieur du corps du parasite Cette organelle que j appelle *campanula* a la base libre et est divisée en deux zones l une interne plus compacte et l autre externe plus claire (Planche XVI figs 1 2)

III —Le troisieme segment constitue le *corps* du parasite proprement dit et montre une structure assez interressante —

(*a*) Plusieurs fibrilles qui peuvent etre divisées en trois séries —

(1) l une constituée par de grosses fibres divergentes plus larges en bas et s insérant en haut sur les granules basaux formant le faisceau inférieur de la double menisque transversale inférieure Ce sont les *myonemmes en eventail* qui forment une sorte de pelerine recouvrant le 5eme ou 6eme supérieur du corps

(2) Ces myonemmes se ramifient et se dichotomisent formant d abondantes *fibrilles obliques* (Planche XVI figs 2 and 4) qui occupent la zone des myonemmes

(3) des *stries longitudinales* dont l origine n a pu etre etudiee avec precision mais qui semblent aussi provenir des granules basaux inferieurs de la double ménisque transversale inferieure parcourent le corps en lignes sousparalleles

(*b*) l ectoplasme est constitué par une mince ligne qui continue la ligne interne juxtaposee au centroblépharoplaste et qui devient plus epaisse vers la partie inférieure du corps,

(*c*) l endoplasme presente deux zones l une externe granuleuse l autre interne alvéolaire se combinant sous des aspects les plus variés des morceaux de bois et des residus alimentaires se trouvant dans la zone alveolaire

IV —Trois series de flagelles recouvrent le protozoaire —

(*a*) la première série sort de la ligne d ectosarque entourant le centroblépharoplaste Ces flagelles sont courts immobiles et traversent la partie correspondente de la *campanula* (*b*) la *seconde serie* se compose de long flagelles extremement mobiles insérés sur les granules basaux du faisceau supérieur de la double ménisque transversale inférieure les plus supérieurs traversent aussi la partie correspondente de la *campanula* mais la plupart deviennent libres sortant par l espace compris entre la campanula et le corps (*c*) la *troisième serie* s insere sur les stries longitudinales Ce sont des flagelles doués d une certaine mobilité semblable a celle des cils des infusoires et recouvrant tout le corps du parasite sauf une petite aire a la partie postérieure

V — Le noyau est situé à des hauteurs variables dans le corps du protozoaire ou il est tout à fait libre et sans aucune formation rappelant la *corbule* ou le *castello* du genre *Trichonympha*. Rond, sa membrane est mince et suivie d'une zone hyaline qui entoure l'endosome proprement dit. Dans celui-ci il y a d'abord à considérer l'existence d'un ou deux granules entourés de vacuole analogues aux *heterochromosomes* décrits par Kofoid et Swezy chez *Trichonympha campanula* et aux *nucléoles* étudiés par Kirby chez *Dinenympha fimbriata*.

La masse chromatique se dispose sur un reticulum de linine plus ou moins apparent prenant des formes plus variées ou souvent on trouve des masses granulaires individualisées ou extrêmement compactes (Planche XVI fig 5 a b f).

Vient ensuite toute une série d'états nucléaires qui rappelent les phenomènes de diacinese décrits par Winiwarter et d'autres dans les prophases des cellules sexuées des métazoaires : noyaux leptotènes, pachytènes et contractions synaptiques (Planche XVI fig 5 d e c). Nous adopterons pour ces états la designa

rencontre à peine le dédoublement du centrobl[illegible]

la disposition de l'endosome suggérant la diacinese, il ne nous repugne pas à admettre que la pseudosynapsis soit un phénomène preparatoire de la mitose nucleaire.

La *mitose* est des plus intéressantes et correspond parfaitement au type général décrit par Grassi et Ana Foà chez le genre *Trichonympha* et plus particulièrement aux descriptions de Koidzumi chez *Pseudotrichonympha grassii*. Malgré que nous n'avons pas trouvé tous les stades, non obstant l'examen des milliers et des milliers d'exemplaires, il y a quelques points qui méritent notre attention : (1) la division commence par le centroblépharoplaste, les deux moitiés étant unies par une *desmose* formant le *fuso externo* de Grassi et Foà (Planche XVII fig 1). L'évolution complète de cette desmose nous est inconnue. (2) On ne trouve pas un synchronisme parfait entre la division du blépharoplaste et celle du noyau : celui-là est souvent complètement divisé et le noyau encore à l'état de quiescence ou le noyau déjà en prophase mais la division du centroblépharoplaste à peine ebauchée (*Planche XVII fig 2*). (3) *on a trouvé une seule fois une figure* semblable à celles etudiées par Koidzumi chez *P grassii* (Planche XVII fig 5) : les centroblépharoplastes fils donnent origine chacun à une fibrille courbe qui vient s'attacher à la *paradesmose* qui présente dans ses deux bouts deux *sphérules* auxquelles suivent les noyaux en télophase avancée. (4) La *paradesmose* a une constitution et occupe des situations variées : ou entièrement sidérophyle et occupant l'equateur (Planche XVII figs 7 8) ou une situation parallele à la direction des chromosomes (Planche XVII fig 6) quelquefois en forme d'une tube hyalin (Planche XVII fig 4) ou fibreux (Planche XVII fig 9) soit une constitution mixte mi sidérophyle mi hyaline fibreuse (Planche XVII figs 2 5). La *paradesmose* est resorbée ultérieurement ; (5) mitose du noyau dont

nous n'avons pas trouve la mctaphse le nombre des chromosomes dépasse legerement le chiffre 12 nombre des chromomères incomptable

VI —*Dimensions* Longueur 180 à 561 microns (on trouve de rares formes jeunes de 80 microns) *largeur* 60 à 221 microns *diamètre du noyau* 17 a 30 *tete* 18 a 21 *centroblépharoplaste* 18 a 22 *flagelles de la lère serie* 20 a 22 avec 5 a 6 pour la pointe libre *ceux de la 2nde serie* 14 à 50 avec pointe libre de 20 à 25, *extremite libre des flagelles de la 3eme série* 8 à 22

VII —*Classification* Les especes de *Pseudotriconympha* décrites par les auteurs sont —

(*a*) *P hertwigi* (Hartmann) Grassi 1911 Noyau dans la portion anterieure Petit nombre de chromosomes 2 a 8 (?) *longueur* 160 (?) 330 à 760, *largeur* 40—80 Par du *Coptotermes hartmanni* Holmgr (Brezil)

(*b*) *P grassii* Koidzumi 1921 *Longueur* 200 à 300 rarement 500 *largeur* 50 a 120 Pas de myonemmes en éventail ni fibres obliques campanula a exister (? fig 18 de la Pl XI de Koidzumi) *sans le developpement de mon espèce* Par du *Coptotermes formosanus* Shiraki (Formosa)

(*c*) *P pristina* (Imms) Cutler 1922 *Longueur* 167 a 280 (Imms) 133 a 259 (Cutler) *largeur* 57 a 144 (Imms) 99 à 226 (Cutler) Campanula et myonemmes non décrits Flagelles de 2 sortes les plus longs de 14 a 16 microns les autres 12 microns Pas de parademose Par de *l Archotermopsis wroughtoni* Desn (Inde)

(*d*) *P sphæropora* Dunkerley Longueur environ 230 largeur environ 67 Presence constante d un ou plusieurs corps spheriques brunatres non colorables plus pales a la peripherie et qui auraient peut etre la fonction d un stercome Par du *Rhinotermes nasutus* Perty (Guyanne Anglaise)

(*e*) *P parvipapillosa* Grassi 1917 Tete centroblépharoplaste tres courts (*e nettamente distinti per la corte a del cape olo*) Par du *Schedorhinotermes intermedius* Braner (Australie)

(*f*) *P magnipapillosa* Grassi 1917 Tete (*assottigl ato*) coiffant le centroblepharoplaste qui est tres large et a la paroi interne *raggrinzita*

Par du *Schedorhinotermes putorius* Cerrakry au pres de la Guinée Française N B Selon Kirby l espece est probablement la meme que *P introflexibilis* Dogiel 1922 parasite du meme termite

(*g*) *P hertwigi var minor Grassi 1917 Les stries longitudinales s etendent* jusqu a la zone de la *tete* la seule organelle qui en est libre Par du *Coptotermes sjostedti* Holmgren (Guinée Française)

(*h*) *P hertwigi var major* Grassi 1917 parasite du *Coptotermes lacteus* Froggart (Australie) Caractères ?

En conclusion l espece du *Leucotermes indicola* differe de toutes les autres jusqu ici connues exception faite de *P hertwigi var major* dont nous n avons pas trouve assez d éléments d identification Nous considerons notre parasite une espece distincte que nous avons intitulee *P bérini* mihi 1927

Comme quelques unes d'elles avaient par moi été décrites auparavant sous le nom générique le *Leidya*, il faut noter ici que j'ai vérifié après que le genre *Leidya* França 1916 est synonime de *Spirotrichonympha* Grassi.

Ceci posé nous avons les espèces suivantes (Planche XVIII).

Espece A. *Caracteres*. Pole antérieur arrondi *sphérique*. *Le faisceau* axostylaire entoure à peine la moitié ou les trois quarts inférieurs de la membrane nucleaire. Les bandes spiralées forment une sorte de carapace enveloppant la partie anterieure de l'endoplasme. Pole postérieur dénude de flagelles dans une étendue variable selon les specimens.

Dimensions: long. min. 70, max. 170; larg. min. 40, max. 145; noyau 12 a 20; pointe libre des longs flagelles 12 a 18.

Décrite par moi sous le nom de *Leidya annandalei* 1918, j'ai en réalité compris sous ce nom deux especes, ne faisant attention qu'à son pole posterieur.

Cette espece differe du *H. hemigynum* Grassi 1917, parasite du *Coptotermes sjostedti* Holmgren (Guinée française), pour avoir le pole antérieur arrondi et par le manque de la zone compacte prenucléaire. Dans la fig. 17 de la tav. VIII de Grassi (21), la seule qui represente l'espèce *H. hemigynum* on ne trouve pas dessiné le faisceau axostylaire, mais je ne retiens pas ce fait comme un caractere spécifique puisque plusieurs exemplaires de ce genre ne montrent pas l'axostyle avec évidence ce qui depend souvent des procédés de coloration.

J'identifie donc cette espece comme espèce autonome et je la nomme *Holomastigotoides annandalei* mihi *sp. N.* syn. *Leidya annandalei* mihi 1918 (*pro parte*).

Espece B. *Caracteres*. Pole postérieur glabre comme dans le type A. Pole antérieur pointu, en cone tronqué, zone triangulaire compacte prenucléaire entourant le noyau dans sa moitié supérieure et s'étendant en largeur souvent plus que la base du faisceau axostylaire qui lui suit.

Dimensions: long. min. 40, max. 140; larg. min. 20, max. 80; noyau 12 à 18; pointe libre des longs flagelles 12 a 18.

Comprise par moi sous la designation *Leidya annandalei* 1918 cette espece ressemble remarquablement le *H. hemigynum* Grassi. La description du Prof. *Grassi étant tres courte il ne m'est pas possible de dire si l'espece du L. indicola* serait une var. *indica* de *H. hemigynum*.

Jusqu'a plus ample informe j'identifie donc l'espèce B comme *Holomastigotoides hemigynum* Grassi 1917 syn. *Leidya annandalei* mihi 1918 (*pro parte*) nec *Holomastigotoides annandalei* mihi décrit cidessus.

Espece C. *Caracteres*. Entierement semblable à l'espece B en ce qui concerne son pole antérieur et l'existence de la zone prénucléaire. Son pole postérieur est pourvu de *stereocilia*.

Dimensions: long. min. 50, max. 90; larg. min. 30, max. 60, noyau 10 a 15, pointe libre des longs flagelles 10 à 18, *stereocilia* plus longs.

Cette espece pourvue de stereocilia avait été decrite par moi sous la designation *Leidya kempi* 1918 qui en realite comprend deux especes. Celle ci ressemble aux espèces *H. mirabile* Grassi 1917 (sauf la fig. 8 de la tav. VIII de Grassi) et

H hartmanni Koidzumi 1921 On peut néanmoins la différentier de *H mirabile* pour avoir les stereocils plus longs et bien plus abondants que ne le laissent soupçonner les dessins de Grassi Grassi remarque aussi que le nombre des spires est constamment 12 tandis que chez mon espèce il est variable Autant que je peux le juger néanmoins on ne peut attacher trop d importance a ce fait comme c est aussi le cas chez le *H hartmanni* de Koidzumi

Mon espece est structurellement egale a *H hartmanni* Koidzumi un peu plus petite que l espèce japonaise (long min 50 max 110 a 170 larg min 30, max 80 a 100 noyau 20 a 26 sur 10 a 15 flagelles 20 à 30 microns)

Je l identifie donc comme *Holomastigotoides hartmanni* var *indica* var *nov* mihi syn *Leidya Kempi* mihi 1918 (*pro parte*)

Espece D *Caractères* P le antérieur en calotte spherique Manque de zone compacte prénucleaire Forme en campanule demispherique Contractions pendant la vie qui peuvent allonger le parasite sans que jamais neanmoins le pôle antérieur prenne la forme d un cone tronqué Flagelles recouvrant tout le corps

Dimensions long min 50 max 90 largeur (à la base) min 30 max 60 noyau 10 a 15 pointe libre des grands flagelles 10 a 18

Cette espèce nommée par moi *Leidya campanula* 1918 reste autonome et devient *Holomastigotoides campanula* mihi 1918

Espece F *Caracteres* Pole antérieur en cone tronqué et avec la zone prénucleaire egale a celle des parasites similaires Forme en bouteille tres caracteristique Periplaste assez rigide Flagelles recouvrant tout le corps

Dimensions long min 50 max 90 largeur (à la base) min 30 max 60 noyau 10 à 15 pointe libre des flagelles 10 a 18

Mes etudes anterieures n avaient pu individualiser ce type La forme du periplaste assez fixe et sa consistence assez rigide me portent a autonomiser cette espece que je nommerai *Holomastigotoides koidzumii sp nov* hommage au confrere japonais Koidzumi

Espece F *Caracteres* P le antérieur un cone tronqué Zone compacte prenucleaire Flagelles recouvrant tout le corps

Dimensions long min 70 max 190 larg min 50 max 135 noyau 15 a 24 pointe libre des grands flagelles 15 à 20

Autonome et parfaitement individualisée comprise par moi sous la designation *Leidya metchnikowi* 1918 qui represente en réalité deux especes je l appellerai *Holomastigotoides metchnikowi* mihi *sp nov* syn *Leidya metchnikowi* mihi 1918 (*pro parte*) nec *Leidya metchnikowi* França 1916 L espèce que j ai decrite en 1918 sous le nom de *Pirsonympha grassii* est la meme que *H metchnikowi* ci dessus

Espece G *Caractères* Conformation semblable à la forme en bouteille du type E dont elle a tous les caracteres Pôle posterieur glabre ayant des *stereocilia* dans sa partie la plus inferieure

Dimensions long min 60 max 100 larg min 30 max 60 noyau 12 à 15 pointe libre des grands flagelles 10 a 15, stereocilia plus longs

Je la considere une espece autonome et la nomme *Holomastigotoides Kempi* mihi *sp nov* syn *Leidya Kempi* mihi 1918 (*pro parte*)

Especе H *Caracteres* Grandes formes allongees plus ou moins cylindr que Pole antérieur arrondi *Manque de zone* compacte prenucleaire Flagelle recouvrant le corps dans toute son etendue

Dimensions long min 150 max 305 larg min 60 max 150 noyau 2 a 24 pointe libre des flagelles 15 a 18

Le plus large des Holomastigotoi les rencontrés chez *L indicola* se caractérisan surtout par un certain sarcodisme du protoplasme en contraste avec les autre formes qui se maintiennent en général inaltérables et pour avoir les spires en *nombre qui tout en subissant des oscillations* est relativement plus petit qui chez les autres especes fait qui devient surtout remarquable si nous comparons l longueur de cette espece et les intervalles entre les bandes beaucoup plus granc qu ailleurs

Je la nommerai donc *Holomastigotoides gigas* mihi *sp nov* syn *Leidya metchni kowi* mihi 1918 (nec França 1916) (*pro parte*)

Structure du noyau a l etat trophique et dans les phenomenes mitotiques

Le noyau n est pas parfaitement arrondi mais elliptique sous globuleux La membrane nucléaire est tres accusés a laquelle se suit une zone plus compacte libre en géneral de tout element chromatique qui se concentre au milieu sous les formes les plus variées tantot des granules arrondis ou ovalaires entassés ou indépendants tantot sous une forme de poussiere ou de fils compacte ou granuleux dont *la structure forme et constitution defient toute description* Entoures de vacuole on trouve un ou deux nucleoles analogues au *heterochromosomes* de Kofoid et Swezy (23) Souvent les granules chromatiques se disposent en 2 masses plus ou moins irregulieres separées et sans que l on trouve entre elles aucune connexion visible fait aussi remarque par Koidzumi chez *H hartmanni* (24)

Viennent ensuite divers états suggérant la diacinese spireme *leptotene* (Planche XIX fig 7) *zygotene* (Planche XIX fig 8) *pachytene* (Planche XIX fig 9) *et contraction synaptique* (Planche XIX fig 5) De tels états sont évidemment préparateurs des phenomenes mitotiques ce qui devient visible lorsque les noyaux synaptiques se suspendent d une tige (Planche XIX fig 14) qui dans les preparations bien réussies fait suite a un point chromatique (Planche XIX fig 12) representant un vrai *centroblépharoplaste* semblable a celui decrit par Kirby chez *Dinenympha fimbriata* (25)

La situation de ce centroblépharoplaste est variable tantot accolé à la membrane meme du noyau (Planche XIX fig 11) tantot au vertex de la zone compacte prenucléaire Peut etre a l état trophique sa situation est intranucléaire et c est lors de la mitose que se fait la migration en dehors la membrane ouvrant une sorte d operculum (Planche XIX fig 14) pour que cette sortie s'effectue

La destinée ultérieure du centroblépharoplaste est son dedoublement (Planche XIX, fig. 13) et la formation du *fuso externo* de Grassi mais cette division peut se faire à l'intérieur même de la membrane nucléaire (Planche XIX, fig. 15). Dans la fig. 12 on montre la première étape de cette division, consistant dans un allongement du centroblépharoplaste.

La division nucléaire est mitotique dont nous n'avons trouvé que peu de figures. Dans la fig. 16 (Planche XIX) on voit un aster, semblable bien que moins développé aux aster décrits par Kirby chez *Staurojœnia assimilis* (26). Dans les figs. 17 et 18 (Planche XIX) on voit dessinée la *paradesmose* creuse et peu sidérophyle, pouvant cependant être compacte et sidérophyle dans quelques exemplaires.

Le nombre des chromosomes semble être 4, devenant diploide plus tard.

La mitose des *Holomastigotoides* autant que l'on peut juger par les figures que j'ai pu trouver appartient donc au type de Grassi.

GENRE SPIROTRICONYMPHA Grassi 1911

Creé par Grassi pour un flagellé erronément classifié par le même auteur en 1892-93 comme une *Pyrsonympha* (27) (sp. *P. flagellata*=*Spirotriconympha flagellata*) parasite du *Reticulitermes lucifugus* nommé aussi *Leidya* par França (1916) (28) et *Cononympha* par Koidzumi: ces denominations devenant donc synonimes. le genre *Spirotriconympha* a les caractères suivants: bandes spiralées devenant transversales a l'extrémité antérieure et laissant libre le pole posterieur. Centroblépharoplaste semblable à celui de *Triconympha* et *Pseudotriconympha*, surmonté d'une petite ampoule et s'étendant jusqu'au devant du noyau où il fait suite a une masse compacte de protoplasme dont le bord posterieur n'est pas tres distincte. Flagelles enfoncés dans le protoplasme surtout à l'extrémité antérieure. Noyau apparemment libre et situé a quelque distance du pole antérieur. Esp. typ. *S. flagellata* Grassi.

Le Prof. Grassi décrit et figure dans l'espece type, ainsi que chez quelques especes exotiques, l'existence d'un faisceau axostylaire auquel Koidzumi ne fait aucune reference.

C'est le moment de signaler deux autres genres tres rapprochés de l'antérieur et qui sont: —

(*a*) *Spirotriconymphella* Grassi 1917: se distingue pour ne pas avoir la partie postérieure dénudée de flagelles et par le manque du *citœndoscleradio*. Esp. typ. et unique *S. pudibunda* par. du *Prorotermes adamsoni*.

(*b*) *Microspironympha* Koidzumi 1921: Noyau attaché au pole antérieur par un centroblépharoplaste. Bandes spiralées provenant de la partie antérieure de cette organelle. Zone compacte prénucléaire entourant le noyau et le centroblépharoplaste. Axostyle (?) Esp. typ. et unique *M. porteri*.

En realité, comme l'ont dejà remarqué les mêmes auteurs, les différences entre ces trois genres sont si minimes que le doute sur leur validité semble justifiable,

d'autant plus que les parasites que je vais décrire semblent représenter des formes transitionelles entre ces genres

Sans rien avancer là dessus je retiendrai pour le genre *Spirotriconympha* ces trois caracteres fondamentaux —

(*a*) *noyau antérieur mais plus rapproché de la zone médiane*

(*b*) spires dexiotropes sortant de l endoplasme entourant le batonnet axial qui situé dans l extremité antérieure est analogue au centroblépharoplaste des genres *Triconympha* et *Pseudotriconympha*

(*c*) zone prénucléaire dont le bord postérieur est peu distincte et n arrive point à entourer le noyau

ESPECES DE *Spirotriconympha* PARASITES DU *Leucotermes indicola* (DESCRIPTION D ENSEMBLE)

Allongés ou plus ou moins arrondis les parasites présentent deux zones l une prénucléaire la seule importante à cause de sa structure spéciale l autre post nucléaire *Celle-ci ne contient que des morceaux de bois et des reserves alimentaires* qui remplissent d ailleurs tout le corps du parasite

Le pole antérieur termine en cone tronque Sur les exemplaires vivants on distingue une petite tete semblable à celle de *Pseudotriconympha* mais qui ne se laisse pas colorer par aucun réactif Couche d ectosarque rigide entourant un *batonnet axial* tubulaire à parois épaisses et refringentes *in vivo* tres sidérophyle sur les préparations à l hematoxyline l extremité antérieure étant constituee par un granule ou baguette assez developpée à laquelle se suit le batonnet axial prenant des formes variées (Planche XX fig 3 a b c d) souvent une ligne compacte souvent deux lignes paralleles ou divergentes ou une ligne centrale et deux ailes latérales plus ou moins sidérophyles

Après un certain trajet le *batonnet* axial se dedouble et limite une zone triangulaire à protoplasme compacte qui tres distincte au debut s efface plus ou moins et tout en se superposant au noyau n arrive jamais à l entourer completement

C est de la zone de l endoplasme entourant le *batonnet axial* que sortent les bandes spiralées (Planche XX fig 4) dexiotropes qui couvrent le corps sauf dans une aire plus ou moins longue au pôle postérieur Nombre des spires variable flagelles tous d egales dimensions sauf les plus antérieurs qui sont un peu plus courts sortant des granules basaux situés dans les sillons creusés par les spires étant enfoncés dans le protoplasme d autant plus profondément qu on se rapproche du pole antérieur

Noyau situé dans la moitié antérieure mais plus ou moins rapproché de la zone mediane en contraste avec la situation tout à fait antérieure du noyau des *Holomastigotoides* La chromatine prend les memes dispositions que chez les autres Triconymphides soit en masses nucléaires soit en figures rappelant la diacinèse (Planche XX, fig 5 a b c d) Le noyau est en connexion avec le batonnet axial par une fibrille (Planche XX, fig 3e) bien visible dans les preparations réussies

La division du parasite d'après les figures que j'ai pu trouver comprend —

(a) phénomènes préparatoires le noyau descendant plus bas que sa situation normale pouvant même occuper la moitié postérieure

(b) le filament moyen du batonnet axial se divise et se rattache à la *paradesmose* de la même façon que chez *Pseudotriconympha grassii* et *belari*

(c) mitose nucléaire Nombre de chromosomes primaires 4, se divisant ensuite pour donner 8

Classification des especes

Deux especes parasitent le *Leucotermes indicola*

(I) l'une plus allongée, l'extremité postérieure dénudée et libre de flagelles dans une certaine étendue variable selon les individus Pole postérieur ovalaire ou fusiforme

Dimensions long min 15 max 52 larg min 8 max 30 pointe libre des flagelles 12 à 18 noyau 6 à 8 microns

(II) l'autre espèce plus courte et trapue les bandes spiralées couvrant tout le corps ou au moins semblant le couvrir tout entier Pôle postérieur large et régulièrement arrondi

Dimensions long min 5 max 30 larg min 8 max 26 pointe libre des flagelles 10 à 16 noyau 5 à 7

Les espèces décrites par les auteurs sont —

(1) *S flagellata* Grassi (1892) 1911 par du *Reticulitermes lucifugus* Italie Portugal possede un faisceau axostylaire qui n'existe pas chez mes parasites

(2) *S flagellata* var *Schedorhinotermitis intermedii* Grassi 1917 par du *Schedorhinotermes intermedium* Brauer Australie flagelles très longs, présence du faisceau axostylaire

(3) *S flagellata* var *Coptotermitis lactei* Grassi 1917 par du *Coptotermes lacteus* Froggatt Australie présente des stries longitudinales sue le corps

(4) *S elongata* Grassi 1917 par du *Schedorhinotermes intermedius* forme tres longue en cigare, noyau situé bien plus bas que dans les especes antérieures et chez mes parasites

(5) *S mirabilis* Grassi 1917 par du *Prorotermes adamsoni* Froggatt Australie Noyau tres proche du pôle antérieur, bandes spiralées occupant seulement la moitié antérieure

(6) *S leidyi* Koidzumi 1921 par du *Coptotermes formosanus* Shiraki, l'ile Formosa Long 15 à 50 microns, largeur 8 à 30 Forme en cone dont la base un peu convexe Flagelles de 10 à 16 microns Noyau au milieu Zone prenucléaire peu distincte auprès du noyau

(7) *S africana* Dogiel 1922 par du *Macrohodotermes mossambicus* Hagen Afrique Orientale Anglaise Ses caracteres me sont inconnus

(8) *S sp* ? (Hartmann) Grassi par du *Coptotermes hartmanni* Holmg Brézil soidisant forme jeune de *Triconympha hertwigi* Ses caracteres me sont inconnus

d autant plus que les parasites que je vais décrire semblent representer d formes transitionelles entre ces genres

Sans rien avancer là dessus je retiendrai pour le genre *Spirotricon jmpha* c trois caracteres fondamentaux —

(*a*) noyau antérieur mais plus rapproché de la zone médiane

(*b*) spires dexiotropes sortant de l endoplasme entourant le batonnet axi qui situé dans l extremité antérieure est analogue au centroblepharoplaste d genres *Triconympha* et *Pseudotriconympha*

(*c*) zone prénucléaire dont le bord postérieur est peu distincte et n arrive po r à entourer le noyau

Especes de *Spirotricon jmpha* parasites du *Leucotermes indicola*
(description d ensemble)

Allongés ou plus ou moins arrondis les parasites présentent deux zones l un prenucléaire la seule importante a cause de sa structure spéciale l autre post nucléaire Celle ci ne contient que des morceaux de bois et des reserves alimen taires qui remplissent d ailleurs tout le corps du parasite

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(b) le filament moyen du bâtonnet axial se divise et se rattache à la *paradesmose de la même façon que chez Pseudotriconympha grassii* et *hilleri*

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CLASSIFICATION DES ESPÈCES

Deux espèces parasitent le *Leucotermes indicola*

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En vue de ces éléments je classifie mes especes de la façon suivante —

(*a*) La première comme analogue à *S leidyi* Koidzumi, en différant cependant par la situation du noyau plus antérieure relativement a l espece japonaise par le notable development du granule antérieur du batonnet et par la connexion visible de ce bâtonnet avec le noyau Je la crois donc une variété nouvelle que j'appelerai *Spirotriconympha leidyi* var *leucotermitis indicolæ* var *nov* mihi 1927

(*b*) La seconde ne ressemble à aucune des especes décrites restriction faite des especes *S africana* Dogiel 1927 et *S sp* du *Copt hartmanni* dont je ne possede pas des descriptions Au cas que la mienne ne soit pas égale a celles ci je appelerai provisoirement *Spirotriconympha rotunda sp n* mihi 1927

Conclusion

Les Triconymphides parasites du *Leucotermes indicola* Wasm sont

GENRE PSEUDOTRICONYMPHA

Espece Unique *P bělări* mihi 1927 syn *Triconympha agilis* mihi 1918 nec Leidy *Holomastigotoides hertwigi* Andrade et Guimarães 1922 nec Grassi

GENRE HOLOMASTIGOTOIDES

Especes

H annandalei mihi *sp n* syn *Leidya annandalei* mihi 1918 (*pro parte*)

H hemigynum Grassi 1917 syn *Leidya annandalei* mihi 1918 (*pro parte*) *nec Holomastigotoides annandalei* mihi (*vide supra*)

H hartmanni Koidzumi var *indica* var *nov* mihi syn *Leidya kempi* mihi 1918 (*pro parte*)

H campanula mihi 1918 syn *Leidya campanula* mihi 1918

H koidzumii mihi *sp n*

H metchnikowi mihi 1918 syn *Leidya metchnikowi* mihi 1918 *nec Leidya metchnikowi* França 1916, *Pirsonympha grassii* mihi 1918

H kempi mihi *sp n* syn *Leidya kempi* mihi 1918 (*po parte*)

H gigas mihi *sp n* syn ? *H mirabile* Grassi 1917 (*partim*)

GENRE SPIROTRICONYMPHA

Especes

Spirotriconympha leidyi Koidzumi var *leucotermitis indicolæ* var *nov* mihi
Spirotriconympha rotunda sp n mihi

INDEX BIBLIOGRAPHIQUE

(1) A D Imms (1919) On the structure and biology of *Archotermopsis* together with descriptions of new species of Intestinal Protozoa and general observations on the Isoptera *Phil Trans Royal Soc* London (B) 209 75 180 pl 3—10

(2) D W Cutler (1920) Part I—Protozoa parasitic in Termites Part II—*Joenopsis polytricha* n gen n sp with brief notes on two new species *Joenopsis cephalotricha* and *Microjoenia axostylis* Quart Jour of Mic Sci 63 555—588, pl 31 33

(3) *Idem* Observations on the protozoa parasitic in the hind gut of *Archotermopsis wroughtoni* Desn Part III *Pseudotrichonympha pristina* *Ibid* 67 247 264 pl 10

(4) Froilano de Mello (1920) Os parasitas multiflagelados do carià na India Portuguesa *Bol de Agricultura* Nova Goa No 1

(5) *Idem* (1920) The Trichonymphid parasites of some Indian termites Report of the Proceed of the third Entom Meeting at Pusa Feb 1919 Vol III 1069 1082 pl 3 Calcutta

(6) *Idem* Sôbre algumas Triconinfidas do intestino do *Leucotermes indicola* Wasm compreendendo uma revisão especial da estampa 51 de Joseph Leidy 1877 Arq Indo Port de Med e Hist Natural No 1 101—136 11 pl (texte aussi en français)

(7) *Idem* Novas pesquizas sôbre os parasitas do *Leucotermes indicola* Wasm *Ibid* 175 199 (texte aussi en français)

(8) *Idem* (1928) Considerations sur les Triconymphides de l'intestin de *l'Archotermopsis wroughtoni* Desn études par le Dr A Imms *Bull de Soc Port des Sci Nat* Lisbon

(9) *Idem* (1919) Note sur les Triconymphides de l'Inde et Ceylan An Scient da Acad Politecnica do Porto

(10) *Idem* (1919) Contribution à l'étude de la faune parasitaire d'*Hodotermes viarum* König de Coimbatore (Inde Anglaise) *Ibid*

(11) C Dobell (1910) On some parasitic protozoa from Ceylon Spolia Zeylanica 7 65 87 pl 2

(12) E Bugnion (1912) Observations sur les Termites Differenciation des castes *C R Soc Biol* Paris 72 1091 1094

(13) *Idem* (1913) La differenciation des castes chez les Termites *Bull Soc Entom* France 8 213 218

(14) *Idem* (1914) *Termes (Eutermes) umbilicatus* Hag *Ann Soc Entom* France 83 39 47 1 pl

(15) *Idem* (1914) La biologie des Termites de Ceylan *Bul Mus Hist Nat* Paris 4 170 204 pl 2 9

(16) E Bugnion et N Popoff (1910) Le Termite à latex de Ceylan (*Coptotermes travians* Hav) *Mem Soc Zool* France 23 105 12? pl 1 9

(17) *Idem* (1910) Les Calotermes de Ceylan *Ibid* 23 124 144 pl 3-4

(18) E Bugnion et C Ferrière (1911) L'imago du *Coptotermes flavus* Larves portant des rudiments d'ailes prothoraciques *Mem Soc Zool* France 24 97—106 pl 2 3

(19) Froilano de Mello (1927) Revision des Triconymphides du *Leucotermes indicola* Wasm Arquiv da Escola Medico-Cirurgica de Nova Goa Fasc 1 Serie A 1 28—8 pl

(20) B Grassi et A Foa (1911) Intorno ai protozoi dei Termitidi *Rend R Accad Lincei* 20 1 sem 725—741

(21) B Grassi (1917) Flagellati viventi nei Termiti *Mem I R Accad Lincei* Série V Vol XII Fasc VIII 1—68 10 pl

(22) J A. GUIMARAES et M. ANDRADE (1922) Note préliminaire su *hertwigi* et l'existenc les *Triconympha* et *Leucotermes indicola* *Congr Med Tro* Medica d'Angola 4,

(23) CH A. KOFOID and OLIVE SWEZY (1919). Studies on the paras Calif Public in 1—116, pl 1—14

(24) M. KOIDZUMI (1921) .. .. Studies in the Intestin of Japan *Parasitol*

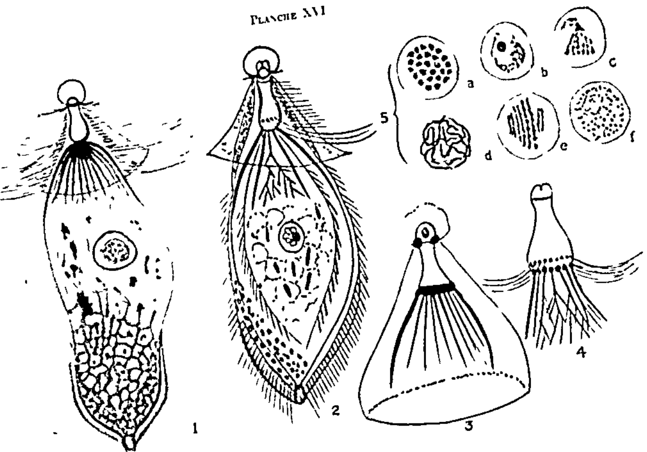
(25) H. KIRBY (1924) .. .. Morphology and Mit *sp nov*. *Univ. Cal* pl 19—22

(26) *Idem* (1926) .. .. .. On *Staurojœnia assimil* from the Termite K 25—102, pl 1—7

(27) B. GRASSI et A SANDIAS (1893) .. Costituzione e svillupo Accad Gioenia Sci I 1 150, pl 5.

(28) C. FRANCA (1916) .. .. Quelques observations *Inst Past*, Paris, 3

PLANCHE XVI



known to those who visited Malay at the Fifth Congress of the Association held in Singapore in 1923

My paper to day will deal mainly with the great conception of our administrator, Sir George Maxwell the third in the direct line of a family who have served Malay with distinction

Sir George Maxwell served his whole life in Malay He interested himself deeply in the health of the people He realized the truth contained in the words of Sir Ronald Ross, The time is one of change and advancement in our ideas of colonial development We are passing away from the older period of incessant wars and of great military or civil dictatorships into one of more minute and scientific administration in which the question always held before us is what can best be done for increasing the prosperity of the people? Sanitation is almost the first word in the answer Prosperity is impossible in the face of widespread disease and perhaps the very first effort which must be done in new countries is to render them reasonably safe, not only from human enemies but from those injuries'

How fully Sir George realized this will be seen from an extract from his paper to the Royal Colonial Institute entitled 'Some Problems of Education and Public Health in Malay' The extract is the part which concerns malaria

Malaria is the curse of the country In the Federated Malay States in 1925 fevers mostly malarial were the cause of 42 per cent of the deaths When I became Chief Secretary to Government in 1921 I found that the Malaria Advisory Board had not had a meeting for some years and had practically ceased to function I revived it and in order to impart to it a certain amount of propulsive force or what some people call 'kick' I made myself chairman This was a purely advisory board and the majority of the members were experts in one subject or another connected with malaria I also established in every district throughout the Federated Malay States Mosquito Destruction Boards which were given complete executive powers and full control over their expenditure votes, and over their staffs and their works The central advisory organization came into close and friendly contact with the district executive organizations and the annual expenditure estimates and programmes of works of the District Boards had to be referred to the Central Board before being submitted to Government for approval This very important provision secured uniformity and co ordination and also often prevented unnecessary expenditure

After careful study of the subject I enunciated three propositions They were as follows —

(1) Every land proprietor is under the burden of carrying out proper and reasonable anti malarial measures upon his land provided that in the case of small holdings and town or village areas the Mosquito Destruction Board may assume the burden, and recoup itself by an assessment

(2) The railway is responsible for railway reservations, and the Mosquito Destruction Boards for all State lands and reservations

(3) In order that anti malarial measures may be effectual there should be co-operation of proprietors of contiguous estates amongst themselves and with the Mosquito Destruction Boards and the Health Officers

The first proposition was entirely new in respect of the liability of the land proprietors for in the past they had carried out only such anti malarial measures as benefited their own employees and had had no regard to anything that was dangerous to their neighbours but not to themselves The proviso relating to small holdings imposed on the Mosquito Destruction Boards a liability which they could take up if they thought fit to do so The second and third propositions stated in clear terms a policy upon which the Malaria Advisory Board had been working since its re constitution, but which it had not yet publicly declared

I put these propositions before the Malaria Advisory Board which recommended them to the Government and later in my other capacity of Chief Secretary to Government I had the pleasure of giving them official approval as the Government policy Since then the Government policy has been widely and continuously advertized I found a convenient opportunity some time later to carry matters to a further stage A Commission was appointed in April 1924 to enquire and advise upon the measures to be taken to improve conditions in regard to health sanitation and prevention of disease on rubber and other estates, upon the system of estate hospitals and nursing and medical attendance therein, and upon the system of visiting estates by medical practitioners

In October 1924 the Commission submitted a careful and useful report with recommendations for improvements upon a co-operative basis in respect of the hospital arrangements and the medical visits It dealt however almost entirely with curative measures and made no proposals for co-operation in anti malarial works When the report reached my office table I drew my attention to this omission in a long covering memorandum and formulated a scheme for co operative system which would include not only the rubber estates but also all contiguous mining lands small holdings State lands and State reservations My scheme was approved by the High Commissioner and a Bill was immediately drafted to give legal force to it After careful discussion with the planters the miners and the private medical practitioners the Bill was passed by the Federal Council last November and became law under the title of "The Health Boards Enactment 1926" The provisions of this law are, briefly There is a Central Health Board with a marked preponderance of unofficials The Board is a body corporate, and appoints a salaried full time Administrator It can employ and pay its own staff of medical officers and can also employ and remunerate the private medical practitioners who have done and are doing wonderful work, both curative and preventive for the rubber estates Local Health Boards are appointed by the British Residents after consultation with the Central Board and are put in charge of specified areas known as Local Boards Areas The Local Board submits to the Central Board its recommendations for co operative curative measures on the estates, such as hospitals dispensaries, ambulances, and so forth, and for the

employment and payment of medical practitioners, dressers, midwives and attendants for visits not only to the estates *but to small holdings*, but also, what is most important of all the Local Board submits its schemes for preventive measures, especially anti malarial works, on all estates, mining lands, small holdings, and State lands and reservations The Central Board may require any scheme to be amended When the scheme is approved it is carried into effect by the Central and not the Local, Board The area to which any scheme applies is known as a 'scheme area' and in any Local Board area there may be dozens of 'scheme areas' whose sizes vary with the nature of the particular problems presented by them

The Central Board has the power to impose an annual cess, or cesses, upon all estates and mining lands inside any 'scheme area' These cesses, which may be separate or consolidated, are collected by the Local Government land officers, and paid by them to the Central Board The convenience, to put it mildly, to the Central Board can easily be imagined The Government pays to the Central Board a contribution at the same rate in respect of all small holdings, and has power to recoup itself, if it wishes to do so by a levy upon the small holders That, however, is no concern of the Central Board, which in any event gets its cheque from the Government When it is remembered that this payment is made by the Government in respect of numbers of small privately owned properties it is difficult to exaggerate the generosity In addition to this, the Government pays, in respect of State lands and reservations, the same cess per acre as is paid in respect of private lands It also pays for the visits of the medical practitioners to the small holdings on the curative work I have already mentioned For a bold, comprehensive, and generous scheme, aiming at the maximum of co operative private enterprise, and a minimum of Government control it would be difficult to find an equal anywhere in the world to this piece of legislation I have, I fear, taken up some time in telling you how it started, and by what degrees it was evolved, and my excuse must be a pardonable pride in my connection with it That it has been possible to introduce this legislation is entirely due to the brilliant work of a number of medical practitioners unconnected with the Government, and wholly employed or remunerated by the rubber estates Of them the best known is Sir Malcolm Watson whose book, 'The Prevention of Malaria in the Federated Malay States,' is a classic on the subject He would, I know, be the first to say that there are many estate medical officers whose successes *in freeing estates from malaria* have been as remarkable as his own I would like to mention some names, but the list would be long, and I should not like to take the responsibility of deciding where to stop The full history of these successes has yet to be written, and I hope that some one will give his attention to it

There is yet a further stage of development, which we have not yet reached in our legislation The law applies only to such small holdings as are included in a 'scheme area' in which there are rubber or other estates There is no provision for a 'scheme area' consisting only of small holdings, or consisting of small

holdings and State lands Such places are now, in accordance with the proviso to the first of my three propositions mentioned already, in the charge of the Mosquito Destruction Boards if they care to assume the burden When the Central Health Board and the Local Health Boards get into full working order, it may be possible to arrange for them to take charge of these places as 'scheme areas'"

In this extract we have the great scheme of a great administrator I venture to prophesy that coming generations will remember the third of the Maxwells chiefly by this enactment and remembering it will count him although last in time, not least in merit

This Health Boards Enactment is among other things nothing less than an attempt to wipe malaria out of Malay And we of Malay may well be asked, how dare we attempt anything so ambitious? That question I propose to answer as briefly as may be

The attempt is possible because Malay has now had 27 years' experience in controlling malaria From small beginnings the work has spread over extensive areas both urban and rural This practical experience has convinced those in control of Malay not only that mosquito control and malaria control are possible but that they are economically desirable and financially practicable Some conditions have made it difficult to control malaria others have materially helped to establish ascendancy over the disease

Climate —In Malay is obviously a most unfavourable factor The temperature is the same to within a couple of degrees throughout the whole year The daily temperature ranges from 71 F to 90 °F The average humidity is 78 and the rainfall from 80 to 200 inches a year every month of which receives enough to keep the grass green and the trees in leaf More favourable conditions for mosquito life could not be imagined

The insects fully realize their opportunity From the point of view of malaria control climate is a real difficulty A very hot or a very dry spell would materially decrease the prevalence of the insect And climate cannot be controlled Not even our most enthusiastic experts have suggested climate control as a means of malaria control

Severity of Malaria in Malay —The states forming the Federation have been under British Protection for only two generations Originally the population was exceedingly sparse and consisted almost entirely of Malayans But with the establishment of peace under the British administration the country gradually at first and later rapidly became opened up Attracted by the high wages of the rubber estates and tin mines Chinese from Southern China and Tamils from South India poured into the country None of these races had any immunity to malaria, with the result that Malay suffered severely from what Christophers and Bentley have described as 'hyperendemic malaria' It meant death rates among labour forces who were not given or would not take, quinine of something well over 100 per 1 000 per annum admission rates to hospital of 3 000 per 1 000 per annum, the practical stoppage of work on the

estates resulting in a luxuriant growth of weeds which made development of the estates very costly How hard malaria can strike was seen at Port Swettenham in 1907 The High Commissioner actually telegraphed an order to close the newly opened port such was the intensity of malaria the demoralization of the services working it and the public outcery against it The disease was no respecter of persons or cases In Kuala Lumpur the capital of the F M S those who suffered most were the highest officials in the administration the best educated the best fed and the best housed in the country

Among the things that favoured the control of the disease I place almost foremost this very *severity* of malaria Where the disease is not very prevalent or not apparently of much importance economically, it is possible to adopt a policy of *laissez faire* to discuss it in a dilettante way, do nothing much to stop it and perhaps never fully realize how much malaria there is, and certainly not recognize the unrecognized malaria' But when, as in Malay the disease is responsible for some 50 per cent of all deaths and at almost every stage thwarts the progress of the country it can hardly be surprising that strenuous efforts should be made to eradicate the pest Money has been available for all well considered schemes The Malaria Advisory Board has been of enormous service to the country in referring back ill considered schemes and in seeing that the country got value for the money spent The F M S is a rich country it has spent money freely on malaria control but what gives me most satisfaction is the knowledge that the money has been well spent and that the country has been enriched by the spending Money spent on malaria control in F M S has been in almost every case money well invested producing enormous dividends The hardest hearted usurer never dared to ask the interest on his loan that money spent on malaria control has repaid freely and voluntarily in cash and in life and happiness in Malay

Research —I would emphasize the front place given to research in the F M S Without it progress would have been impossible The invaluable researches carried out in other countries have been studied carefully I refer in particular to the work done in Africa and India by Daniels Stephens Christophers James and Bentley and to the work of Gorgas Darling and le Prince in Havana and Panama elsewhere by the Rockefeller Foundation The F M S itself has not been idle The Institute for Medical Research was started in Kuala Lumpur in 1899 with Hamilton Wright as its first Director He published its first volume of 'Studies' in 1901 on Malaria and Mosquitoes He was succeeded by Daniels Fraser, Stanton and Fletcher, they all published researches on malaria or mosquitoes Leicester of the Institute published in 1908 a large volume with the title the 'Culicidæ of Malay'

In 1912 the *Malaria Bureau* was started in Kuala Lumpur with Strickland as its organizer and first research officer He was succeeded by Hacker, Lamborn and Williamson All four added materially to our knowledge while Strickland made a discovery of first class importance in the prevention of the disease, a

discovery that to-day is enshrined in the law of the Straits Settlements, and one that is constantly kept before the public of the F M S by the warning notices of the Malaria Advisory Board

Species Sanitation —From research came the policy of species sanitation, of vital importance in the rural districts of Malay where there is so much rain and water that one wonders at times why all creatures have not developed webbed toes This species sanitation led us to distinguish different zones of land, and to adopt appropriate measures for each *One man's meat is another's poison* In Malay a method successful in one zone may be a deadly danger in another Research showed us why taught us the correct methods to use and how to avoid danger

Experiment —Early in the history of malaria control in Malay, the value of experiment was understood The use of experiment is perhaps the feature that most distinguishes modern scientific work from that done in the Middle ages One has only to read the history of how great discoveries have been made to realize how slow even the greatest minds have been in seeing into the future and of what in comparable value to them have been their experiments in leading them to the truth Those who have inherited the truth stand almost aghast as they watch the master minds groping in the dark, working their way by experiment towards the light, seemingly quite incapable of jumping forward to the conclusion that they ultimately reach, which to those who follow seems to be inevitable if not from the start of the work at least in the later stages Well may one of the most distinguished workers in medical research emphasize the value of technique and experiment and the comparative worthlessness of the empirical methods' Let me quote from him 'Empirical methods take cognizance only of what comes without our going in quest of it into our field of experience, and they take into account only that knowledge which is brought to us directly by our five senses In other words, in empiricism we have that which unregenerate man most desires, an evangel which prescribes all delving below the surface of things, all going in quest of knowledge all employment of apparatus, and all troublesome technique, in short a gospel which holds out promise of knowledge unpurchased by arduous labour'

Experimental research has therefore taken a foremost place in Malay As malaria control to be effective must be done over a considerable area, so our experiments have been carried out on many acres of land As the years have passed, our technique has improved Our methods of measuring the amount of the disease before, during and after the experiment have been improved Exact observations have been made on how the various species of insects have been affected, as various deliberate alterations have been made on their environment As the physician has called the chemist and the instrument maker to his aid to evolve the science of bacteriology and the control of bacterial diseases so in the control of malaria he has called in the *entomologist* and the *engineer* By their aid malaria control in Malay, which began in small urban areas in 1901, extended to a wide rural area in 1905. In 1911, a new *technical method* for drying up ravines was used on Seafield

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Among the things that favoured the control of the disease I place almost foremost this very *severity* of malaria. Where the disease is not very prevalent, or not apparently of much importance economically, it is possible to adopt a policy of *laissez faire* to discuss it in a dilettante way, do nothing much to stop it, and perhaps never fully realize how much malaria there is, and certainly not recognize the 'unrecognized malaria'. But when, as in Malay, the disease is responsible for some 50 per cent of all deaths and at almost every stage thwarts the progress of the country, it can hardly be surprising that strenuous efforts should be made to eradicate the pest. Money has been available for all well considered schemes. The Malaria Advisory Board has been of enormous service to the country in referring back ill considered schemes, and in seeing that the country got value for the money spent. The F. M. S. is a rich country, it has spent money freely on malaria control but what gives me most satisfaction is the knowledge that the money has been well spent and that the country has been enriched by the spending. Money spent on malaria control in F. M. S. has been in almost every case money well invested producing enormous dividends. The hardest hearted usurer never dared to ask the interest on his loan that money spent on malaria control has repaid freely and voluntarily in cash and in life and happiness in Malay.

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Estate, in Kuala Lumpur and in Singapore a method which our *engineers* have developed and refined to the admiration of all who have seen it Of course it has not all been plain sailing Experimental work never is We have had *many failures* and many difficulties I could give many instructive examples, the failure in Kuala Lumpur described by Dr Wellington, but for details these I must refer you to my 'Prevention of Malaria' I will however mention one on Terentang Estate in Negri Sembilan An experiment was begun by the Malaria Advisory Board in 1913, but the technique was not good Only now in 1927 has it been perfected, so that reliable observations may be made and final conclusions drawn

Of enormous importance to the country have been the *Mosquito Destruction Boards* They have made exact observations on malaria and mosquitoes, and have devised and drawn up scientific schemes for the control of the disease Not of least importance has been the training they have given to the Subordinate Health Staff in the recognition of species of Anopheles in the larvæ stage in the making of malaria surveys, and in the supervision of anti malarial work Generous help has been given to many estates by the Staff of the Mosquito Destruction Boards, and to day by advertising one can obtain without much difficulty men who are familiar with the microscope and with anti malarial work To Dr Wellington and his staff the F M S are under an obligation they can never repay

The Mosquito Destruction Boards and the Estates where malaria control is well organized will be the centres from which control will be spread over the whole country Of course it will take time to get the New Health Boards organized, but by another ten years there will be great, if not spectacular, progress

Mosquito Control —Many methods will be used chief reliance will be placed on *mosquito control*, by jungle cover, jungle clearing drainage, oiling, etc Quinine as a prophylactic *has proved a complete failure As a cure I have a profound faith in it*, if the patient takes enough and for a long enough period It has been my fortune, good or bad, to have been infected three times with malaria, twice *with benign tertian and once with sub tertian All have been severe attacks* All have promptly subsided under quinine I have taken 21 grains of quinine bihydro chloride daily, rarely missing a dose, for three periods of six months, five months, and six months, respectively The drug has not caused me the slightest inconvenience Meyer's reagent showed it was well absorbed No relapses have occurred during or after the treatment In fact, I felt particularly fit when taking the drug and sometimes almost imagined I had become—what, in the case of quinine seems an impossibility—an 'addict' Yet despite both my preaching and my practice I find it difficult to persuade others to continue the drug for long enough to prevent relapses and my faith in any general population taking the drug for many days after the attack is past is nil Even if we had a drug so effective that it would give an absolute cure in three days, we would be exactly in the position of those who have to deal with yellow fever—powerless to control the disease in the presence of even comparatively small numbers of the efficient insect carriers. Think for a moment of the struggle in Panama to stamp out yellow

[illegible] and [illegible] satisfactory [illegible] answer [illegible] that is after [illegible] months of hard work [illegible] nothing except lack of [illegible] [illegible] of the entire labour force writes Mr. [illegible] the secretary of the [illegible] in Malaya and [illegible] [illegible] And in these [illegible] statistics show that however [illegible] the labourers [illegible] the death rates and sick rates are always [illegible] than [illegible] among the people [illegible].

Cost of Malaria Work. [illegible] the discovery of the [illegible] in certain [illegible] malaria [illegible] land which [illegible] [illegible] could be [illegible] jungle. [illegible] Sometimes the costs [illegible] A recent example is the case of a large company where [illegible] on anti-malarial work [illegible] the company [illegible] £[illegible] sterling a month. [illegible] to the company [illegible] capital of £[illegible] sterling and [illegible] to the [illegible] wages on account of sickness, etc.

At the other end of the scale there are anti-malarial costs that make the [illegible] [illegible] and through [illegible] about £1 10 sterling per 100 feet [illegible] in Malaya and that is a permanent [illegible]. The capital cost of subsoil drainage is heavy in Malaya but spread over 20 years [illegible] depreciation and a sinking fund [illegible] less than [illegible] drainage system [illegible] £20 per head of the population per year.

It is a true way [illegible] that labour is very expensive in Malaya and that malaria can generally be controlled for the amount an Indian labourer can earn by three days' work. This sum enables the labourer to work on many days when, but for the malaria control, he would be too ill to work. It means profit to the labourer; and where the worker is the owner it means an abundant profit, wealth, prosperity, a happy and healthy family. Knowing these things Malaya spends money freely on malaria control, and means to spend more in the future on a system that will spread health all over the country. For we have the faith which is a sense of conviction and set with the sharp [illegible] of experience. And we have a deep faith that experimental research will greatly cheapen our methods in the future.

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fever, and how near to failure the Americans were in 1906 that is after 18 months of hard work in controlling the *Stegomyia* nothing except lack of sailing accommodation prevented the scattering of the entire labour force' writes Mr Bishop the secretary of the Canal Commission Only in very small communities in Malay and where mosquito control is physically or financially impossible do I use quinine alone And in these places statistics show that however healthy the labourers may appear to be the death rates and sick rates are always three or four times higher than normal—although being in small communities the few deaths that occur cause no comment among the people themselves

Cost of Malaria Control—This varies enormously Strickland's discovery of the harmlessness of certain jungles and of the value of shade in certain zones gave us a method of controlling the most virulent malaria in hill land which cost *absolutely nothing* Research again showed us that malaria on flat land could be completely avoided by selecting sites for houses half a mile from undrained jungle The selection of a non malarial instead of a malarial site costs nothing Sometimes the cost may be quite small—we cast a sprat to catch a whale A recent example is the case of a large company—where under £100 a month spent on anti malarial work will save the company and the contractors together close on £20 000 sterling a month by preventing delay in the completion of the work avoiding loss to the company of interest and profit on a capital of £2 000 000 sterling and loss to the contractors under the headings of overhead charges and increased wages on account of sickness etc

At the other end of the scale there are anti malarial costs that make the sanitarians of poor countries shiver in despair Upkeep of open drains and thorough oiling in intensely malarial hill land costs about £1 10 sterling per 100 feet per annum a startling figure when one remembers the mileage oiled in Malay and that it is a recurrent expenditure The capital cost of subsoil drainage is heavy in Malay but spread over 20 years even with full depreciation and a sinking fund it is less than one third of the cost of oiling Oiling an open drainage system in land much cut by ravines, may cost as much as £20 per head of the population per annum

But a truer way of reviewing the figures is to remember that labour is very expensive in Malay and that malaria *can* generally be controlled for the amount an Indian labourer can earn by three days work This sum enables the labourer to work on many days when but for the malaria control he would be too ill to work It means profit to the labourer or where the worker is the owner it means an abundant profit wealth prosperity a happy and healthy family Knowing these things Malay spends money freely on malaria control and means to spend more in the future on a system that will spread health all over the country For we have the faith which is woven of conviction and set with the sharp mordant of experience' And we have a deep faith that experimental research will greatly cheapen our methods in the future

When I had the honour of opening the discussion on malaria at the Congress at Singapore in 1923 I used these words —

'We often talk of the campaign against malaria To day I would suggest another simile which perhaps more correctly suggests our position at this time We have hardly begun the great campaign against malaria yet I would say that Laveran found the ore Manson and others sketched the furnace Ross built the furnace, smelted the ore and gave us the pure metal It has been our duty to forge weapons from the metal and to test the worth of the different weapons for, as in actual warfare, more than one kind of weapon is required

In the past twenty years we have been scouting rather than fighting skirmishing with the enemy to find his strength and weakness He holds the ground with unequal strength in different parts When we have found out these things we may then plan a great campaign and press it with confidence in the event '

In opening the discussion to day in Calcutta, in 1927, the position in Malay is different The great campaign has begun The head quarters staff is working at full pressure Mobilization is in full swing Battalions are being brought up to war strength and new battalions enrolled We will strike cautiously, but courageously Confident in our careful training for the fight, with a knowledge of the strength of the enemy but not discouraged by it, prepared for a long and hard campaign we shall press on, assured of a great victory

REMARKS ON ANTI MALARIAL MEASURES FOR POVERTY STRICKEN REGIONS

BY

LIEUT COL S P JAMES, M D, I M S (RETD)
British Ministry of Health, London

THE following remarks on anti malarial measures are concerned only with the malaria problem in Europe and only with the problem in areas where very little money is available I think no excuse is necessary for confining them to the European problem because that is a subject which, up to the present, has not received the attention it deserves As regards the limitation to malarious districts where very little money is available, that is a limitation rendered necessary by the circumstances in which malaria occurs as an endemic and epidemic disease in Europe Let me give an example At the present time Bulgaria is endeavouring to make arrangements for the prevention and control of malaria among nearly two million peasants of Bulgarian nationality who have returned to their own country as refugees from Macedonia, Thrace, Yugo Slavia and Asia Minor since 1912 These refugees, consisting of about 32,000 families, are mostly homeless and without land They must be settled in rural districts and the only way in which that can be done is to distribute them in and around existing villages most of which unfortunately are already very malarious The majority are being settled in the Government of Burgas where the average spleen rate is over 40 per cent, about 75 per cent of the enlarged spleens reaching nearly to the umbilicus The breeding places of the malaria carrying Anopheles are exceedingly numerous and extensive, consisting of lakes, swamps, borrow pits, mountain streams, rivers and irrigation ditches At present in these villages the only assistance available in cases of sickness is such as can be given by the village schoolmaster or priest who is provided by the Government with a supply of quinine to be distributed to anyone who may ask for it For the purpose of settling refugees on the land, the Government, backed by the League of Nations, has obtained a loan of about 2¼ million pounds, which is about £1 per head of the number of refugees concerned This loan is required to be repaid in 20 years by the refugees themselves with interest at 7 per cent. In order to place the refugees in a position to earn the bare necessities of life and to pay the interest on the loan, practically all the money available must be spent on reclaiming land so as to make it suitable for cultivation, and on providing

houses agricultural implements, seed and cattle When this has been done little money will be left even for ordinary medical assistance and any expenditure on preventive measures which may not be immediately productive of material results in money or kind is hardly to be thought of

There are problems of the same kind and with similar financial difficulties in Italy Serbia Greece Roumania Albania Russia and other countries of South Eastern Europe In malarious areas of those countries there are many people who from lack of means are obliged to live in huts which are little better than the huts of primitive man and there are people who have no hut of their own but live (as Celli has described) like modern troglodytes in caves excavated in the rocky hills or like nomads in make shift tents

Now it has to be admitted that in circumstances of poverty such as those to which I refer there is not and perhaps never will be enough money to apply the methods of malaria control which have proved effective in certain small and relatively wealthy areas in various parts of the world No one doubts the efficacy of those measures when they can be thoroughly applied, but everyone agrees that they are difficult and very expensive

Therefore it is immensely important to endeavour to discover a method of dealing with malaria which can be effectively applied with the small amount of money that is usually available in the type of malarious districts to which I have drawn attention

In May 1923 the Health Committee of the League of Nations appointed a Commission *whose task it is to endeavour to solve this problem for Europe* The Commission is an international group of malariologists and public health officers Most of its members are workers in Europe but the membership also includes Dr Chagas of Brazil Dr Raynaud of Algeria and Col Christophers of British India *Their inclusion does not mean that the mandate of the Commission* extends beyond Europe I think I should make this quite clear by saying that the object of the tours of enquiry of the Commission in Palestine the United States and one or two other countries outside Europe has been solely to gather experience which may be useful for the solution of the European problem

Up to the present the Commission has published two general reports several special reports on particular study tours in different countries and one laboratory report In the second general report a summary is given of the present views of the Commission on measures for dealing with malaria in Europe The Commission has not yet succeeded in finding a simple and cheap method of dealing effectively with the disease in poverty stricken districts They believe that the best prospect of success in this quest lies in a renewal of activity in the research of malaria in all its aspects In the report mentioned an endeavour is made to bring this view to the notice and urgent consideration of European governments and two methods of enquiry which might be profitably pursued are suggested

But the Commission does not for a moment contemplate the cessation of anti malarial efforts while that research is being pursued Therefore the main part

of the report is concerned with suggesting to the European governments concerned the measures which seem justifiable in the present stage of knowledge and experience In the time allotted I can only deal briefly with these suggestions in a general way They are based on the view that because no royal road nor short cut to the prevention of malaria by breaking one of the links of the epidemiological chain has yet been found the wisest course that European countries with limited funds can adopt at present is to continue to combat the disease itself on its appearance in the human and insect hosts As regards the disease in the human host it is advised that the first aim should be to reduce its severity rather than to aim immediately at reducing its incidence The results of the Commission s enquiries seem to show that when attention is directed chiefly to reducing the severity and duration of malarial attacks rather than to reducing incidence, the disease soon ceases to be of importance from the public health point of view This phenomenon is seen in North Holland There is still quite a considerable incidence of malaria in that country but local study will convince you that as an appreciable factor in the state of the public health the disease long ago lost all its importance A similar result has come about unconsciously in several other European countries and in many parts of the United States of America In these places the disease was robbed of its importance without any reduction of Anopheles mosquitoes and in some places even before the role of the mosquito was known As regards the disease in the insect host it is the case in Europe that malaria infected mosquitoes are found almost exclusively inside human dwellings and usually indeed only in dwellings where a member of the household is suffering from the disease Therefore the Commission considers that the systematic killing of blood filled mosquitoes which can be found in the interior of dwelling houses should everywhere be attempted

Both the above measures are classified as direct Among indirect measures the Commission attaches most importance to agricultural and industrial welfare schemes which aim at improving the economic and social conditions of the people and their general well being and standard of life The Dutch polders' and the Italian bonifica are schemes of this kind They are not concerned with the reduction of mosquitoes Their object is primarily social—to change a poverty-stricken sparse scattered often semi nomadic population into one which is settled and well to do, with proper arrangements for housing water supply education and general welfare and with adequate medical attention A change of this kind does not eradicate the causes of endemicity and the sources of malaria but it quickly brings about a cessation of severe and fatal cases and a significant reduction of bad effects so that the disease comes finally to be of little or no importance as a cause of sickness and death

Anti larval measures in the general environment are classed by the Commission as a very indirect method of attempting to deal with malaria The Commission does not doubt that in Europe the present abundance of *Anopheles maculipennis* can be materially reduced in some localities by anti larval measures persistently

carried out in accordance with the most modern methods but during all their journeys in different countries they found only a very few localities in which it could reasonably be hoped that those measures could be prosecuted with any hope of obtaining sufficient success to warrant the large staff and great expense that would be necessary even for a limited campaign Therefore they hope that in most malarious localities of Europe the cheaper and less difficult anti malarial measures which they suggest will suffice to bring about the limited result towards which they think the malarious countries of Europe should aim

MALARIA—MOSQUITO CONTROL IN RURAL SINGAPORE

BY

J W SCHARFF B A M D D P H D T M & H

THE island of Singapore contains an area of 217 square miles The municipal area is 29 square miles in extent the remainder termed 'rural' is principally agricultural land interspersed with villages tenanted partly by field labourers and, to an increasing extent by town workers The excellent results of anti malaria work within urban limits have been recorded by Dr Hunter the Municipal Health Officer The extension and adaption of anti malaria measures to rural districts beyond the town is the subject of this paper

ADMINISTRATION

The administration of anti mosquito measures in Singapore is controlled by the Government and Municipal Health Officers in their respective areas These officers are the sanitary authorities acting by virtue of an anti mosquito ordinance under the provisions of which anti malaria measures are carried out

Funds are provided by the Municipality from general taxes in the Municipal area and by Government from revenue in the rural area

No special improvement rate is levied on the lands that are freed from malaria but the law provides that owners of property shall if they have the means to meet the expense, pay for the cost of anything that may be required to free their land from mosquitoes In practice I have found it difficult and unfair to extract payment for anti mosquito oiling or drainage work except under estate conditions Landowners who possess unproductive swamps near villages naturally object to paying for improvements that benefit others only

The amount recovered for anti malaria work done in private property in the rural area amounts to less than 6 per cent of the total outlay on the campaign In towns or in populous village areas anti malaria work should be regarded as a health measure benefiting the whole population and should as far as possible be financed and maintained as such disregarding possible benefits to individuals In respect of finance emphasis need not be laid on the riches of Malay Money is as hard to obtain for health work there as anywhere else in the world and it is only because anti mosquito work is proved to be a paying proposition that the Government and the public alike support it

INVESTIGATIONS AND STATISTICS

The anti malaria campaign to which I refer was preceded by preliminary investigations lasting about one year directed towards the study of statistics,

There were 46 children under 12 years of age in this village at the beginning of that year and of these 42 had enlarged spleens The deaths recorded from Bukit Timah village during the year totalled 45 and this number calculated on the mid year population of 459 is equivalent to a death rate of over 90 per thousand There were only four babies born and of these two died of malaria within six months of birth

The population remained numerically the same new arrivals to the village barely kept pace with deaths and the departures of the sick

This is no isolated example of the ravages of the disease but it was in this case accompanied by an active campaign of quininization aided with the usual propaganda including lectures and cinema demonstrations

It is probable that the failure of quinine was due to the fact that the amount consumed fell short of the actual requirements of the population but I must emphasize that quinine was distributed as widely as possible Quinine sulphate in mixtures and in 5 grain capsules was also stocked and issued free of charge at schools and police stations The consumption was at the rate of 3 lbs per month in Bukit Timah or approximately 9 600 daily 10 grain doses costing annually $324 or nearly 70 cents per head per annum whereas the average cost of effective malaria mosquito control by appropriate measures in villages is a mere fraction of the money which would be required to dose the population with quinine *continuously*

The detailed results of eradicating the malaria carrier in this locality are shown in Table I

The Distribution of Local Anophelines

Early in my investigations of the rural area I found that the places from which malaria patients were admitted to hospitals and the areas with high spleen rates amongst children corresponded very closely to the extent of the breeding places of *Anopheles maculatus*

In contrast to the intensity of malaria in such areas some localities both inland and along the seashore were entirely free from malaria in spite of the presence of vast numbers of Anophelines chiefly *Anopheles vagus A kochi* and *A hyrcanus* *Three very restricted breeding places of Anopheles ludlowi exist on the seashore and this mosquito is responsible for some malaria there* A study of local conditions bearing upon the incidence of malaria shows that the presence or absence of a single species of Anopheles is the determining factor of the presence or absence of malaria *It seemed certain that neither drugs nor ordinary measures of sanitation* were of any avail in combating this virulent species but that the remedy lay in attacking that dangerous local species in its larval state a stage at which it is most readily destroyed

This plan of *attacking the offending species is based upon the knowledge that* the larvæ of different species of mosquitoes are adapted to live only in certain kinds of water Thus in dealing with *Anopheles maculatus* we have a mosquito the

larvæ of which can only thrive in well ærated spring water arising as a rule from a granite formation When these mosquitoes are deprived of suitable breeding places they rapidly die out It has been suggested that in the course of time these mosquitoes may adapt themselves to other conditions but this has never in my experience been the case This presumption is based upon the observation that during the progress of anti malarial operations *A maculatus* larvæ are sometimes found breeding in unusual situations such as cement wells and water tubs but I have only been able to find temporary and isolated instances of such adaption to an unsuitable environment and they have had no effect upon malaria control Larvæ of *Anopheles maculatus* discovered by one in unlikely breeding places numbered altogether 216 and of these seven reached the pupal stage and two emerged whereas the average number of *A maculatus* larvæ collected and identified yearly in the laboratory amounts to 2 350 specimens of which 15 per cent normally hatch out

In carrying out anti larval measures the field worker and possibly those who finance our present methods of control are constantly distracted by the idea that some cheaper method of malaria control is in progress of being discovered Fascinating excursions can be made into the field of biological control but fortunately in Malay though the need for research is not overlooked there is a demand for immediate action and the only measure at present effective is the eradication of the dangerous species either by anti larval poisons or by drainage

Anti larval Poisons

Oiling mixtures or poisons such as Paris Green when used in the field are inevitably washed away or rendered inactive as soon as they have killed the existing larvæ

Paris Green has so far only been tried experimentally in Singapore I consider that the great objection to its use is its invisibility when dusted on water surfaces Owing to this the work of efficient supervision over the unreliable labour which is the only kind available is infinitely greater than the supervision required over large areas controlled by oiling for oil leaves clearly visible effects on vegetation Another local objection to Paris Green is that it is difficult to handle in wet weather and in a country where rain is a prominent feature throughout the year, this is a serious objection to its use In cases where we deal with a mosquito whose virulence as a carrier of malaria is less marked than that of *Anopheles maculatus* and where interruptions of control measures through rainy weather are of less consequence as is the case in an attack against *Anopheles ludlowi* then Paris Green has an undoubted value

Oiling versus Drainage

In view of these local conditions therefore I shall discuss the value of species control by means of drainage in contrast to oiling control both of which measures have been widely employed in the rural area

The spraying of mosquito breeding places with oil is regarded as essentially a temporary measure and to be effective it must be repeated weekly throughout the year over an area of half a mile from the outskirts of the village zone By exercising careful control over the staff employed in oiling and by intelligent anticipation of possible new breeding places for the dangerous larvae there is a rapid disappearance of malaria Such supervision on the large scale required for scattered villages embracing many square miles even though we enjoy the advantage of visible oil on the surface is too great a tax upon the energy of the officer in charge Spraying entails the constant transport of a heavy material and coolies are always on the alert to pour oil in bulk down a drain to relieve themselves of the unwelcome burden

It is evident therefore that the more breeding places are permanently removed the greater will be the area over which malaria control can be efficiently carried out with a combined system of oiling and drainage

The combination of these two methods of control secure thoroughness and permanence and have been the means of eradicating malaria from a large section of Singapore Island

We can never afford to neglect the use of oil spraying as a temporary measure in combination with drainage but it has been my experience that drainage applied only to such places where dangerous mosquitoes can breed is ultimately not so expensive as the cost of the oil that is used over long periods It should be possible to employ these measures in malarial places with similar local conditions if the population is sufficiently numerous within the village zones to render such work financially reasonable

The essential details of the simple form of drainage required can be learned by any anti malarial officer success depends upon carrying underground in pipes the particular type of water wherein *Anopheles maculatus* breeds at a sufficient depth to avoid choking of the pipes with roots

Examples of the cost of drainage in comparison with the cost of oiling have been worked out in a number of different localities and in each of these the capital cost of draining is between three to five times the annual cost of oiling The maintenance of drainage is a comparatively small item and the security from malaria is infinitely greater in consequence of the elimination of the unreliable human factor The ultimate saving is therefore obvious since the cumulative cost of oiling overtakes the initial cost of drainage within the space of a few years

Subsoil drains once properly graded and laid with well baked tile pipes should remain effective for very many years provided that simple precautions are taken with regard to keeping deep rooting vegetation clear of the pipe line

By means of drainage swamps are reclaimed and land is brought into a condition suitable for agriculture

The water in subsoil pipes can be put to various uses For instance near Bukit Timah I have used the supply from drains for maintaining an even flow of water into a septic tank which treats the sewage of the entire population of the village

Elsewhere, supplies of drinking water have been provided in specially constructed wells on the line of subsoil pipes Care is taken to enhance the value of drainage from the point of view of the public by such means as these

Some engineering knowledge is required for drain construction, and here is a difficulty that all health officers or scientists engaged in the practical application of species sanitation are likely to have to face

The training and ambitions of the expert engineer does not ordinarily lead him to devote time to the study of the habits of different species of mosquitoes, to consider the effective range of these insects or to interest himself in the minute details necessary for a complete scheme of species control It is only rarely that an engineer will willingly subordinate his public works activities to those of public health Construction of roads bridges and buildings, by reason of their greater cost, naturally claim closer attention than relatively inexpensive drainage measures I have to stress this subject because in Singapore where there is no anti malaria engineer schemes for drainage and all details of administration are entirely in the hands of the health departments This is a novel procedure but one which may with advantage be adopted elsewhere, if that rare individual the anti malaria engineer is not available By anti malarial engineer I mean a man who is employing his whole time and energy upon public health The importance of unity of control in measures directed towards the improvement of public health is exemplified by the need that exists for intimate co ordination between temporary relief measures such as oiling, and permanent anti larval measures, such as drainage A correct perspective in public health measures generally must moreover be maintained, with the ultimate object of securing the maximum benefit for the minimum cost This can, in my opinion, be best achieved if all such measures are directly controlled by the department responsible for public health This is spoken in a spirit of humility, for, in their own special spheres we must still look to the engineer, the chemist and the biologist, for assistance, advice and co-operation

Rural Organization

The essential feature of anti malaria measures in rural Singapore has been the organization of district health units There are five sanitary districts, each approximately 60 square miles in extent All public health measures, with the exception at present, of child welfare, are in direct charge of a fully trained sanitary inspector, resident in each district A district store and coolie lines [illegible] established and a campaign of oiling dangerous breeding places within half a [illegible] radius of each village area is begun being followed by gradual ext[illegible] of permanent drainage schemes within that area

The central supervising and laboratory staff consists of one chief [illegible] inspector, a qualified drainage inspector and two surveyors a laborat[illegible] and three mosquito collectors The control work is principally [illegible] mosquito surveys Spleen surveys, malaria case records and vital [illegible] district also provide material upon which the progress of malaria [illegible]

The Cost and the Result

An annual vote of $100,000 has been available for rural anti malarial work since the year 1922, and in carrying out this policy of oiling and draining during the past six years, more than 56 miles of subsoil pipes and 8 miles of open concrete channels have been laid and a yearly average of 18,000 gallons of oil have been sprayed, protecting an area of some 15 square miles

To the end of the year 1926, approximately $320 500 have been spent on anti malaria work The cost of maintenance and temporary work during 1926 amounted to $17,538 The population protected from malaria numbered approximately 39,300 Estimating the cost from this population alone the capital cost of *malaria control averages $1 65 (3s 8d) per head* per annum, while maintenance of existing works and oiling cost 45 cents (1s) per head per annum The relative costs within the municipal area of Singapore with its denser population is at the rate of only 25 cents (6d) per head and 4 cents (1d) per head for maintenance cost

The following map shows, approximately, the localities where malaria control measures have been carried out, both within the municipal boundary and in the rural area of Singapore, and also illustrates the localities that have not yet been freed of the dangerous malaria carrying mosquitoes

There is evidence of a steady improvement in the prosperity and health of the inhabitants in villages where anti malaria work has been undertaken There has also been an increase in land values, to which this work has in no small measure contributed The record of the improvement in Bukit Timah village during the period under review has been as follows —

Table I

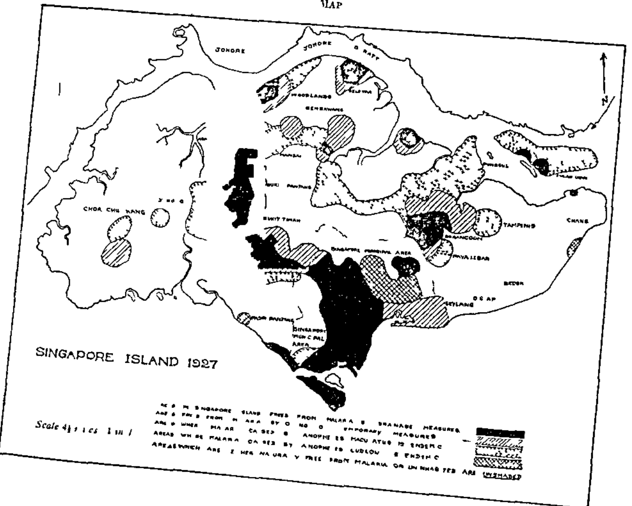
Bukit Timah Village Statistics 1921 to 1923 and 1926 and 1927.

Year	Number of children examined	Number with enlarged spleens *	Spleen rate per cent	Mid year population	Malaria patients	Deaths recorded from malaria	Total deaths	Births
1921	49	43	87 7	459	180	38	45	4
1922	*53*	*39*	*73 5*	*487*	*102*	*28*	*31*	*3*
1923	64	15	23 4	510	78	6	8	16
1926	72	6	8 3	632	69	9	16	11
1927 †	75	4	5 3	680	57	7	12	22

* The spleen rates are those recorded for the month of June each year

† Refers to the period January to October

MAP


JOHORE
JOHORE
WOODLANDS
SEMBAWANG
CHOA CHU KANG
BUKIT TIMAH
TAMPINES
CHANGI
PAYA LEBAR
BEDOK
GEYLANG
SINGAPORE MUNICIPAL AREA
SINGAPORE ISLAND 1927

The record for the whole of rural Singapore, which may be divided into areas according to the present distribution of malaria, is tabulated below

TABLE II

ear	Areas where anti malaria measures have been in force and extended since 1921 (Bukit Timah Bukit Panjang Woodlands Paya Lebar Serangoon and Pasir Panjang)			Districts which are normally healthy and where no control measures are required (Siglap Bedok and Changi)			Villages and kampongs which are malarial and where no control measures have been instituted (Ponggol and Jurong)		
	Number of children examined	Number with enlarged spleens	Spleen rate per cent	Number of children examined	Number with enlarged spleens	Spleen rate per cent	Number of children examined	Number with enlarged spleens	Spleen rate per cent
921	222	74	33.3	154	6	3.8	22	8	36.3
922	259	57	22.0	266	22	8.2	16	5	31.2
923	347	52	14.9	69	3	4.3	31	16	48.3
926	509	19	3.7	254	18	7.0	65	61	93.8
927	126	7	5.5	120	7	5.8	48	39	81.2

Two villages and some sparsely populated country, which is in the *maculatus zone and in which it has not been possible to undertake anti mosquito measures* have served as a control The population of these places is estimated at 11,180

An epidemic of malaria due to climatic conditions which favoured principally *Anopheles maculatus* occurred in many parts of Malaya in the year 1926, there was however, no infection of malaria traced to places where *maculatus* control measures were effective, whereas in other places, notably the villages of Ponggol and Jurong in Singapore the increase in the spleen rates of children, and the mortality due to malaria was very considerable

A large section of rural Singapore, and some of the most populated areas containing some 32 100 persons is naturally free from malaria, with the exception of small endemic foci near *Anopheles ludlowi* breeding places on the coast The breeding places of *Anopheles ludlowi* so far as they exist in the rural area, are relatively of little importance owing to their small extent, and because of the scattered population in their immediate surroundings This is in contrast to the extent of the breeding places of this mosquito in the neighbourhood of the tidal creeks upon which part the town of Singapore is situated The control of *Anopheles ludlowi* by depriving the larvæ of suitable breeding places is being undertaken at present, and is no less practicable than the effective control of *Anopheles maculatus*

Conclusion

The success which has attended the thorough eradication of the dangerous mosquito carrier in rural Singapore leads me to hope that similar measures of species sanitation may be applied to village areas in other parts of the tropics As I have shown the cost is insignificant compared with the benefits conferred on the public in general both in health and prosperity

THE THEORY AND PRACTICE OF MALARIA 'CONTROL'.

BY

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CONTENTS

I PRELIMINARY REMARKS

IT is unnecessary before this audience to dwell upon the fact that the 'control' of malaria constitutes one of the biggest if not the biggest public health problems confronting the administrator and the sanitarian in the tropics

It is not proposed to quote statistics in support of this statement more especially as mortality rates where malaria is concerned, do not fully reflect the state of the public health but when the moral, physical and economic degradation associated with paludism is taken into account, there is little doubt that the well-being of an absolutely large proportion of the inhabitants of the tropics is more or less gravely compromised by the malaria parasite

No apology is therefore necessary for the present address upon the subject of the Theory and Practice of Malaria 'Control'—but the title of the paper may perhaps call for a brief word of explanation

To some the problem of malaria 'control' was solved some thirty years ago when Sir Ronald Ross working in the heart of this city, completed his great *discovery of the part played by the mosquito in the spread of the disease and it* is now widely believed that all or nearly all there is to learn about malaria is known and that what is now required is not discussion, however illuminating or investigation, *however interesting, but serious and sustained effort to extirpate* the mosquito and to banish malaria from a 'fever' stricken world

To those who hold these views a discussion of the problem of malaria 'control' will appear superfluous, but, as some regard our present *methods of 'control' as*

falling short of perfection an analysis of the present position in the light of modern knowledge and experience may perhaps serve an useful purpose

India has no startling achievements to record in respect of malaria 'control' and it may therefore appear that an Indian worker who presumes to speak upon this subject invites the rebuke administered by Ophelia to her brother

> Do not as some ungracious pastors do
> Show me the steep and thorny way to heaven
> Whilst like a puff'd and reckless libertine
> Himself the primrose path of dalliance treads
> And recks not his own rede

In extenuation I can only hope that some account of the experience acquired during the course of a prolonged pilgrimage along the steep and thorny way to malaria control may be the means of eliciting the views of those entitled to speak with authority upon this important subject

These are my apologies and I must now crave your indulgence whilst I briefly describe the present position as I conceive it of this complex problem

I think it will be agreed that a discussion of this subject is peculiarly opportune at the present time in the first place thanks almost entirely to Sir Ronald Ross whose absence from this Congress owing to ill health is a grievous disappointment, the malaria problem is attracting public attention at the present time to an extent without precedent secondly the presence of Sir Malcolm Watson Sir Ronald Ross fidus Achates and of distinguished representatives of the Health Committee of the League of Nations in the person of Dr Madsen of Lieut Col S P James of the Ministry of Health London of Professor J W W Stephens of Liverpool, and of many whose names are household words amongst those acquainted with the modern literature of malaria provide an unique opportunity of comparing notes and of exchanging experiences

II The Theory of Malaria 'Control

(a) *Quinine Medication*

The first to attempt the control of malaria were the aboriginal inhabitants of Peru to whom the curative properties of cinchona bark had probably been known centuries before the year 1638 when the miraculous recovery of the wife of the Spanish Governor of Peru (the Count of Chinchon) was the means of bringing its virtues to the notice of the civilized world

This discovery was made in the complete absence of any knowledge of the mode of action of the bark and the bark was exhibited on frankly empirical grounds but it nevertheless represented the first of a series of approximations in the long and chequered history of malaria control

The second approximation was due to the French chemists Pelletier and Caventou who in the year 1820 isolated from Jesuits bark the quinine and other alkaloids upon which it is now known that its medicinal properties depend The

may be not anti-larval measures or even quinine medication, valuable adjuncts though they may be, but the institution of measures to ensure that the labour *force is properly housed and more especially properly fed* These may be extreme instances but it can scarcely be doubted that measures calculated to raise the economic status of communities constitute an important aspect of all operations designed to 'control' malaria

The importance of the human factor has not always been accorded practical recognition by tropical sanitarians, but improvement of economic status constitutes the basis as well as the essence, of the method of 'bonification' by means of which, in the absence of anti larval measures, a considerable measure of 'control' has been achieved over malaria in Italy. May it not be that the disappearance of malaria in England, in the absence of any attempt at mosquito 'control' or of the systematic exhibition of quinine, is in a large measure attributable to the fact that scarcity and famine as the result of bad harvests, are no longer apt to occur in that country and is *it not probable that malaria was the result rather than the cause of the decline* of ancient Greece ?

Be this as it may, the study of malaria in the Punjab has led to the conclusion, to which as the second Report of the Malaria Commission of the League of Nations shows European malariologists also subscribe, that measures designed to raise the social and economic condition of a people constitute anti malaria measures of profound importance and it is doubtful whether any anti malaria campaign which fails to take the human factor into account can be regarded as in complete harmony with scientific requirements

(ii) *The Transmission Factor* —The transmission factor is usually regarded as embracing the carrier insect alone and it is customary to appraise the insalubrity of malarious localities solely in terms of the prevalence of Anophelines The syllogism has, in fact gained wide acceptance that water means mosquitoes, mosquitoes mean malaria therefore water means malaria and, in consequence, anti malaria measures have come to be regarded as almost synonymous with anti larval measures But does this view represent the whole truth ? The mosquito passes only one relatively short stage of its life history in water and it is surely inexpedient to ignore the adult insect and to fail to take into account the circumstances conducive to the *acquirement* and to the *transmission* of malaria by the Anopheline mosquito

The biological method of malaria 'control' envisages the adoption of measures calculated not only to destroy mosquito larvæ but also measures designed to reduce the power of the mosquito to acquire and to transmit infection It is a fair criticism of what may be termed the 'pure water school' of malariologists, not that they have attached too much importance to the mosquito—it would be difficult to do so—but that by confining attention to the immature insect, they have unduly restricted the scope of anti malaria measures

It is furthermore clear that mosquito 'control' is not a *sine qua non* of malaria 'control' It is only necessary to refer to the phenomenon of 'Anopheles *sine* malaria,' one instance of which is the large measure of 'control' achieved over

malaria in Italy by the method of 'bonification,' in spite of the fact that this measure has actually led, in some instances, to the increased prevalence of Anophelines These facts therefore suggest that it may be possible to devise means whereby some measure of 'control' can be achieved over malaria without necessarily obtaining complete 'control' over the mosquito

It is to the elucidation of this problem that attention has been mainly directed in the Punjab during the past 13 years, and laboratory studies, combined with field investigations, have led to the conclusion that measures designed to modify the environmental conditions affecting the adult insect may in certain circumstances constitute anti malaria measures of the first importance

Nothing is more striking in the Punjab than the absence of relationship between the relative prevalence of Anophelines and the local incidence of malaria and nothing is more conspicuous than the relatively high incidence of malaria in association with environmental conditions characterized by relatively high atmospheric humidity The outcome of a prolonged study of the influence of atmospheric temperature and humidity upon the power of the mosquito to acquire and to transmit infection permits of the conclusion that the association between malaria and marshes and between pools and paludism, hitherto regarded as almost solely dependent upon an abundance of water collections must be largely ascribed to the influence of environmental conditions upon the power of the adult insect to acquire and to transmit infection The same remark applies to the close association often found to exist between excessive vegetation and a high local incidence of malaria

The practical implications arising out of these studies are of far reaching importance The object of anti malaria measures being primarily the 'control' of malaria and not necessarily the extirpation of the mosquito it is clear that measures designed to prevent flooding, to lower the level of the sub-soil water, to improve land drainage, to remove excessive vegetation in the vicinity of human habitations, are calculated, even although they do not directly lead to the destruction of mosquito larvæ, by reason of their influence upon atmospheric humidity, to play an important part in reducing the incidence of malaria

Time does not permit of a more detailed reference to the subject, but sufficient has been said to indicate the nature of the biological method of malaria control Much investigation remains to be carried out before all the possibilities of the biological method of control are exhausted, but it is even now clear that it broadens the basis of 'control' measures and provides new methods of combating the disease

III The Biological Method of Malaria 'Control'

The principles underlying the biological method of malaria 'control' are of universal application, but in practice they necessarily require to be adapted to local circumstances and conditions

So far as the Punjab is concerned the conclusion has been reached, for reasons already given, that anti larval measures (by means of existing methods) combined

made during the past 300 years is the *precise measure of the advance made in our knowledge of the epidemiology of the disease*

The present position would appear to be that science has placed at our disposal various methods by means of which some measure of 'control' can be achieved over malaria Quinine medication has its value but the simplest and most effective method if it be practicable, is the complete extirpation of the mosquito It is the simplest method because it does not involve any extension of existing knowledge and it is the most effective method because if there are no mosquitoes there can be no malaria But the combined experience of many workers gained during the course of some 20 years in many tropical and sub tropical countries suggests that malaria control by means of existing methods is not always practicable and it must *therefore be concluded that the methods now available represent temporary* expedients an approximation to an ideal not yet attained—rather than the last word of Science upon this subject

(c) *Biological 'Control'*

Huxley if not the first was certainly one of the strongest advocates of the view that a definition of terms and a reversion to fundamentals was periodically *necessary in all branches of natural science, and it may therefore be appropriate* to apply this maxim to the problem associated with the attempt to sever the age long association between man and the malaria parasite Now it is clear that the disease malaria represents the objective phenomena occasioned by the invasion of the human body by the malaria parasite *It is likewise clear that the objective signs* of the disease are in some measure dependent upon the intensity of the infection (*the parasite factor*) and the degree of resistance of the human host (*the human factor*) The spread of infection is however determined by the occurrence of circumstances favourable to the transmission of infection and hence *a transmission factor* must also be taken into account

Three factors—the human factor the parasite factor and the transmission factor—must thus be regarded as concerned in the production of every malarial infection and it therefore follows theoretically at any rate that the control of malaria may be encompassed either by measures designed to render the human host resistant or refractory to infection or by measures that will destroy the parasite *in the tissues of the human host or by measures that will sever the link* between the human host and the reservoir of infection

It is obvious that measures having any one of these objects provided its sovereign efficacy be unquestioned will suffice for our purpose and that alternatively some or all of them may be required in order to enable partial or complete 'control to be obtained This method which may be termed the biological method of 'malaria control thus envisages the employment not of one or even of two measures but of all measures calculated to sever the association between man and the malaria parasite

Let us now consider the possibilities attaching to this method of malaria 'control'

(i) *The Human Factor* —Take, for example the human factor

No method has hitherto been discovered of rendering man partially or completely refractory to infection with the malaria parasite—the so called immunity exhibited by adults in hyper endemic areas has obviously been purchased at too great a price—but malaria being a disease which tends in the absence of repeated infection to die out spontaneously it appears to follow that measures designed to raise the resistance of the human factor must be regarded as one method of attempting to achieve a biological 'control' over malaria

That the human factor plays a prominent part in the endemiology of the disease has long been recognized S R Christophers and C A Bentley were perhaps the first to stress the great importance of this factor more especially in connection with the occurrence of malaria amongst labour forces in the tropics The important part played by famine in the natural history of epidemic malaria in the Punjab was also elucidated by Christophers whilst Bentley has long maintained that the malaria problem in Bengal is essentially an economic problem The same view is implicit in the aphorism of E L Perry that malaria is a disease of waste land waste water and waste people

The scientific study of malaria in the Punjab during the past fourteen years has served to emphasize the profound importance of the economic factor both in respect of endemic and epidemic malaria Time does not permit of a detailed reference to the result of these investigations * and it must suffice to state that wherever scarcity prevails as the result of water logging excessive salinity of the soil or of long continued agricultural depression a high degree of endemic malaria (hyper endemic malaria) almost invariably prevails Investigations carried out in these localities have shown that the high incidence of the disease cannot be attributed to any peculiarities of climate to an unusual parasite to a strange insect vector or even to an abnormal abundance of Anopheles (which indeed are often not more prevalent in hyper endemic areas than in adjoining healthy areas) and the inference is unavoidable that the associated economic stress plays a predominant part in determining the high endemicity of malaria in these areas

This illustration therefore serves to suggest that measures designed to remove the cause of economic stress constitute anti malaria measures of considerable importance It is indeed clear that in certain circumstances anti larval measures even in association with the exhibition of quinine if not accompanied by measures that will raise the economic status of the community may be almost valueless Similarly as Christophers has shown in connection with the tropical aggregation of labour when non immunes are imported into a malarious terrain and placed under highly adverse economic conditions the most important anti malaria measure

* An account of these investigations is given in The Genesis of Epidemics Baillière Tindall and Cox London

next great advance was the discovery by Laveran in the year 1880 of the malaria parasite Empiricism now gave place to exact scientific knowledge and it was permissible to infer that the disease malaria was caused by a specific parasite and that the curative properties of cinchona bark were attributable to the parasiticidal action of its alkaloidal 'content'

We do not yet know precisely how *quinine acts in malaria and even if, as some* one has put it we still pour drugs of whose action we know little into bodies of whose action we know less yet all are agreed that the cinchona derivatives have at present no rivals as a means of curing and more especially of mitigating malaria

Nevertheless valuable although quinine medication undoubtedly is few are now prepared to hold that the eradication of malaria upon a large scale even if the world supply of quinine were sufficient for the purpose, can be achieved by means of quinine medication *alone*

(b) *Anti Mosquito Measures*

The drainage of marshes and even the use of mosquito nets was practised upon empirical grounds centuries before the year 1897 when Sir Ronald Ross proved that the Anopheline mosquito played an essential part in the life history of the malaria parasite but it was not until this fundamental fact had been established upon a scientific basis that the possibility of achieving 'control' over malaria upon a large scale came to be envisaged as even a remote possibility

It followed in fact, as a natural implication of this classical discovery, that complete 'control over malaria could be achieved by means of the extirpation of the insect carrier and henceforth malariologists kept one auspicious eye upon quinine and the other upon the mosquito

It would serve no useful purpose to recall the great argument that raged 'about it and about' a decade or so ago and it will suffice to state that some pinned their faith upon one measure and some upon the other and a few upon a judicious combination of them both The policy advocated by Sir Ronald Ross has however prevailed and it is now generally held that the mosquito rather than quinine constitutes the key to the solution of the problem of malaria 'control' It was not unnatural in the first blush of the successful discovery of the role played by the mosquito in the spread of the disease that optimistic views should have been formed in regard to the possibility of achieving a dramatic victory over malaria by measures directed against the mosquito

The striking success attending the use of these measures—anti larval measures in association with various forms of mechanical protection and the exhibition of quinine—at Ismailia, in the Panama Canal zone and in the Malaya States—served to confirm the accuracy of these views and it was assumed that similar methods could everywhere be applied with similar results But 'experience is deceitful and judgment is difficult' and the point for consideration at the present time is not whether the extirpation of the mosquito is an effective means of controlling

malaria—the instances quoted above provide an answer to this question—but whether measures that can be applied with success under certain special conditions—where the malariologist exercises 'control' over man in the shape of a labour force and a not less effective 'control' over the money bags—provides a practicable method of eradicating malaria upon a large scale at all times and in all places

In many countries it has been found that the difficulties of *obtaining* and *maintaining* 'control' over the mosquito are extremely formidable and attempts have consequently been made to limit 'control' measures to the species locally concerned in the spread of the disease ('species control') and, as a further application of this principle, it has recently been suggested by S P James that anti mosquito measures should be limited to what may be termed 'specimen control' or to the destruction of those insects (possibly or probably infected) found in human habitations

These attempts to find a new approximation that will render mosquito 'control' more effective and less costly have served to emphasize the now well established view that, before pronouncing an opinion upon the practicability of anti mosquito and more especially of anti larval measures it is necessary to study the local problem

In the case of Europe, the Malaria Commission of the League of Nations in a recent pronouncement state that in the unanimous opinion of a number of distinguished European malariologists, anti larval measures do not constitute the most practicable and perhaps even the most effective method of 'controlling' malaria in this continent The administrative financial and technical considerations that have led European malariologists to adopt this view probably apply *a fortiori* to many countries in the tropical and sub tropical zones, each area however, requires to be examined upon its merits, but so far as the north of India is concerned, no one acquainted with the conditions prevailing in the Punjab during the malaria season—the monsoon period—can fail to be impressed by the magnitude of the problem presented by an attempt to eradicate malaria by means of existing methods of mosquito 'control' When one takes into account the climatic conditions prevailing during the monsoon period, the habits and customs of the people, the nature of their homes (90 per cent live in small mud built villages) the physiographical features and the character of the soil—a featureless plain readily flooded by even a few inches of rainfall—the innumerable water collections in and around every village, and the innumerable Anophelines in every homestead, it is difficult to avoid the conclusion that the men, money and material are not available in India to cope with a problem of these dimensions by means of existing methods

To sum up the history of malaria 'control' thus briefly outlined, shows that an initial frank empiricism has gradually given place with the advance of scientific knowledge, to increased precision and increased efficiency in the methods of 'controlling' malaria It is a far cry from Peru to Panama and the measure of the progress

with quinine medication do not provide a practicable means of eradicating malaria in this province This statement must however not be regarded as an admission that India in general and the Punjab in particular has been treading the primrose path of dalliance during the past two decades

On the contrary an anti malaria policy based upon the biological method of malaria control has been gradually evolved and brought into operation

An attempt has thus been made to control' malaria by measures based upon the human factor the parasite factor and the transmission factor

So far as the human factor is concerned, it may properly be held that the 10 5 million acres under canal irrigation constitute from the malaria point of view a vast bonification' scheme since although canal irrigation may have enabled two Anopheline larvæ to grow where one grew before canal irrigation has banished the spectre of famine increased the wealth and prosperity of the Punjab and has raised the standard of living When the important influence exercised by economic stress upon the human factor is taken into account and when the effect of the prosperity resulting from canal irrigation in permitting a vast increase in the number of schools of hospitals of roads and of improved methods of agriculture is also realized and when it is mentioned that an immense impetus has been given during the past three years to these and other beneficent activities on the personal initiative of His Excellency Sir Malcolm Hailey, the Governor of the Punjab it is impossible to avoid the conclusion that much has been done to dissipate the malaria complex—an inferiority complex—and to reduce the incidence and intensity of both endemic and epidemic malaria

Canal irrigation was however, not introduced as an anti malaria measure and an increase of malaria is still regarded by many malariologists as the price that must be paid for freedom from famine and for an assured food supply Canal irrigation has indeed in some areas been responsible for water logging but this condition whose evil effect upon agriculture and upon health is equally great is the result not only of seepage from canals but also of spill water from rivers and mountain torrents and of excessive rainfall

To deal with these problems a Drainage Board now termed the Rural Sanitary Board was created in the year 1919 and this body is now engaged in the execution of measures designed to prevent flooding to improve land drainage and to lower the level of the subsoil water

Six great drainage projects are now under construction either directly by the Rural Sanitary Board or by the Irrigation Department at a capital cost of Rs 27 65 679—(£212 744) which when completed will drain an area of approximately 2 000 square miles by means of some 200 miles of land drains

In order to deal with the water logging problem a research laboratory in charge of an expert attached to the Irrigation Department was opened two years ago and at a recent conference His Excellency the Governor announced that neither money nor effort must be spared in the endeavour to provide an effective remedy for this evil In the meantime reclamation work has been started in one hyper endemic

area of 3 000 acres where the Irrigation Research Officer is experimenting with various methods of drainage with a view to reducing the water table and to restoring the fertility of the soil

Canal irrigation has also been reduced in the vicinity of towns and villages and lift irrigation (in some instances by means of tube wells worked by electric power) has been installed in several localities

The draining of swamps and the levelling of depressions on the outskirts of towns has also been carried out more especially at Amritsar where open parks and pleasure gardens now exist in places where ten years ago the soil was permanently water logged

Finally special attention is being given to the removal of jungle growth and excessive vegetation from the vicinity of human habitations and the question of modifying the method of irrigation within municipal limits is under consideration

In the case of the parasite factor a scheme for taking a spleen census of school children was inaugurated in the year 1914 and this scheme as the result of which some 80 000 children in some 900 localities are examined for splenic enlargement twice a year has been in continuous operation for the past thirteen years but the complementary scheme drawn up in the same year whereby all malarious scholars were to be placed upon a course of quinine has for various reasons not yet fully come into operation On the other hand a scheme for the free distribution of quinine in rural areas through the agency of District Medical Officers of Health is now in operation in addition to the scheme for the sale of quinine through Post Offices

Finally mention must be made of the Epidemiological Bureau formerly termed the Malaria Bureau which came into existence in the year 1910 where the scientific work upon which the biological method of malaria control is largely based has been carried out

IV Conclusion

To sum up it is held as a result of this brief analysis of the malaria problem that so far as the tropics generally are concerned no single existing method of control can be regarded as providing a basis upon which the eradication of malaria upon a large scale can everywhere and at all times be achieved It must furthermore be concluded that quinine medication larval control and other existing methods represent finger posts along the road to absolute knowledge and that it is necessary if any further advance is to be made along this road to seek a new approximation based upon a fuller knowledge of the natural history of the disease The final solution of the malaria problem would thus appear to depend upon patient and searching investigation and careful and continuous experiment It may be that an epoch making discovery will provide some short cut to victory but in the absence of a discovery of this nature no dramatic conquest of malaria would appear to be possible in the near future It would rather seem that the control of the disease may in many parts of the tropics best be achieved by the slow operation of all types of measures that will on the one hand *increase resistance of the human host* and upon

water in sewers, when for want of water the sewers do not operate as such The question in reality is how is a water carriage sewage system to be worked without water My reply is that Madras must solve that problem for itself Wells can be kept free from larvæ by stocking them with fish, but the people of Madras must be educated not to eat the fish

Col James's paper —I have heard with the deepest interest and I have also studied the second General Report on Malaria in Europe of the League of Nations If the titles were altered to 'Anti malarial measures, exclusive of anti larval control' I think they would be much more appropriate With much in their report I am in complete sympathy but I feel that unless anti larval control is stressed much more than is done in the League's report the ultimate result of the League's report will be to mislead Europe into neglecting the measure which we, in Malaya have found most effective and will lead to profound disappointment From my long experience of the beneficial effects of larval control, even in small communities, it is unthinkable that a fruitful breeding place for the larvæ of a malarial carrier should be left in the centre of a village and the population advised to wait for the benefits which are to come, in the possibly far distant future from 'bonification,' improved housing or any other indirect measure

I cannot too strongly express my dissent from a policy which would advise the neglect of anti larval measures in such conditions and I am sure that if Europe adopts such a policy on the League's advice it will ultimately bitterly regret it I wish to associate myself with the detailed criticism of the report made by the Hon'ble Dr Hoops, and I regard Major Hitchens suggestion that a portion of the money given to the people in Bulgaria should be ear marked for anti malarial work as one of great value, and one which should receive the careful consideration of the League

Lieut-Col S P James, I M S (retd) (Great Britain) replied It is interesting and helpful that at this discussion the subject of anti malarial measures has been treated from two entirely different points of view Sir Malcolm Watson and Dr Hoops have described the measures adopted in certain small wealthy areas in Malaya and I have drawn attention to the problem in some large, poverty stricken areas in Europe The circumstances and conditions of *the examples cited are so different that it would be* surprising if the same anti malarial measures were applicable to both Therefore, I feel that Dr Hoops must be under a misunderstanding if he is dismayed because our recommendations for Europe differ from his recommendations for Malaya Malaria control of course, is a local problem and the anti malarial method of choice is the method best suited to the local conditions, there is no known method which can be described as being superior to all others and therefore as being applicable everywhere This being so, each country is free to choose the particular methods of malaria control to be adopted, and each country (and to a more limited extent each locality) must 'work out *its own salvation* '*in this matter These* are some of the principles upon which stress is laid in the report of the Malaria Commission of the League of Nations and I do not think they indicate that the Commission favours either the 'wet school' or [illegible] school' of malariologists to which reference has been made They indicat[illegible], in the opinion of the Commission, the European co[illegible] concerned s[illegible] an open mind on the

subject and should not adopt a particular anti malarial policy on the ground that it is believed to have been successful in some other country where conditions may be quite different It would not be correct to say that the Commission is more in favour of intensive quinine treatment as an anti malarial measure than it is in favour of anti larval measures The Commission is unanimously of opinion that quinine has no effect in preventing infection by the bites of infected Anopheles , also that, however carefully quinine may be used in routine practice its effect is chiefly to lessen the fatality, severity and duration of attacks rather than to reduce the number of cases Anti larval measures also possess serious defects it does not seem necessary to enumerate them or to endeavour to decide whether in general they are fewer or more numerous than those attending the use of quinine What seems to be much more important is to cease from exaggerating the merits of either measure Instead we should in my opinion tell administrators and sanitarians quite plainly that we do not, as yet, possess any single or simple method of malaria prevention or control capable of application in all malarious districts and that for this reason what is really needed is renewed activity in research and the intensive study of the disease in all its aspects Continued persistence by certain schools of anti malarial practice and opinion in the old time belief that the discovery of the mosquito cycle of the malaria parasite did in fact provide sanitarians with a unique, practical and definite solution of the problem, has greatly hindered and delayed this research and has made it more difficult to obtain funds and workers to conduct it While it is being pursued it is wise, in my opinion, to refrain from advising poverty stricken countries to undertake costly and ambitious schemes which may appear theoretically to have a high scientific value , we should, instead, restrict particular anti malaria measures to those which are obviously beneficial and immediately practicable, and we should concentrate attention upon building a permanent foundation of all round medical and sanitary arrangements upon which special campaigns against particular diseases, including malaria, may ultimately be based

(*This* Discussion *is continued on page* 748 —ED)

May I request the members of the Congress to let me know the method or methods by which mosquito breeding can be prevented in underground sewers especially in a city like Madras where there is scarcity of water ?

Dr Victor G Heiser (U S A) In order that the members of the Congress may be able to judge of the relative capacity of the people of Bulgaria and of Malaya to pay for malaria control measures I should like to ask Col James what data were used in coming to the conclusion that Bulgaria could not afford to pay what Malaya finds possible ? The basis of the amount of taxes might serve as a guide What is the total per capita tax in Bulgaria as compared with Malaya ?

Dr S K Ganguli (Bengal) The conclusions of the Malaria Commission as outlined by Lieut Col S P James, in his opening paper and the observations made by Sir Malcolm Watson lead malaria stricken Bengal nowhere, as both of them seem to be pessimistic about the conquest of the scourge by the administration of quinine and they are doubtful if quinine can cure malaria or prevent its occurrence, although it is claimed that it can reduce the severity and incidence of the disease to a great extent Bonification of the soil and people is urged The actual parasite its host and the parasiticide drug, quinine, have been discovered and there is no division of opinion as to this amongst the experts It is gathered from the discussions that there is no single method of malaria control which is best for every locality The topographical condition and geographical position of Bengal is such that she requires a special method of prophylaxis to eradicate the malady Bengal is a land of rivers streams and pools and there is a sufficient natural provision of water ways Destruction of larvæ and prevention of their breeding is regarded by certain experts as one of the anti malarial measures Drainage may not be regarded as an anti mosquito measure It is, however, believed by the people and certain schools of thought that no scheme can be worked out successfully if the natural water ways and water courses are not attended to The question of 'dying rivers' 'high roads' and 'railways' should therefore, not be left out of consideration by scientists engaged on malaria control Again, the country is faced with acute mass poverty and mass illiteracy and it appears to me that no preventive measures can succeed so long as attention is not directed to the economic problem and mass education, because education is the solution of many ills The moot point is the financial question The experts have got to see if disease prevention should precede or accompany disease cure and that the money spent over both is sufficient Control of malaria is, to my mind impossible if adequate money is not found for it Larvicides may be prohibitively expensive but it is for the Congress and the League of Nations to find out a cheap and, at the same time an efficient prophylactic for the guidance of the Governments and the peoples committed to their charge Perhaps further research and investigation may be called for The functions of this Congress, I believe, do not end in merely throwing out suggestions, but by recording their votes also as to whether the scientific findings are properly applied for the benefit of humanity in India The unified efforts of the Government and the people, supported by 'brains and wealth,' and extensive propaganda to educate the mass are needed to win the victory over malaria

Dr A R Wellington (F M S) Col James has dealt with malaria control in certain countries of Europe under conditions which appear to be entirely different from

those prevailing in Malaya I can offer no criticism of the methods proposed for the European countries for I feel sure the various methods of control were carefully considered before that report was written

In Malaya experience has shown that quininization will not effect any improvement on an estate severely infected with tropical malaria Immunization will in time come about and the health *of those remaining improve but this state is only reached after* half the population or more has succumbed We have not been able to teach our people to hunt for mosquitoes in their houses and to kill them In some estates mosquito nets *have been given out free and the coolies refused to put them to their proper purpose* and used them rolled up as pillows With such conditions we cannot expect any improvement from mosquito destruction in houses The net result of 27 years of trial is that we believe the anti larval method is the best for our country

We have done a great deal but we believe we can do more Up to date certain malarious estates have done a great deal but there are some which have done practically nothing To even up this state of affairs the Health Boards Enactment was framed It was originally called the Estates Health Board Enactment but on redrafting the word estates' was dropped as it was hoped to include areas within flying distance of estates i e Kampongs and small holdings I do not share the optimism of Sir Malcolm Watson that the enactment will in the near future eradicate malaria from such places as remote Kampongs though perhaps in the end these areas will be dealt with

Dr C Strickland (Bengal) May I ask whether Col James can give any definite figures showing the benefit of bonification ?

We have some experience in the Dooars of North Bengal Here in 1908 Christophers and Bentley took the splenic indices in about 20 tea estates and in 1926 I took them on the same estates—the difference was almost nil although the welfare of the coolies had been improved out of all knowledge in the intervening time The malaria sickness there is still extremely severe

Prof J W W Stephens (Great Britain) While congratulating the wet school — the anti larval school—on the success they have achieved I think they have not done themselves complete justice in that they have not always recorded their failures for I suppose everybody admits that there have been failures and a study of the cause of these would be instructive and would probably lead to the avoidance of particular methods I confess I have leanings towards what may be termed the dry school those who advocate the destruction of the infected mosquito or what may be termed comprehensively the anti parasite school For it is evident that if the parasite can be destroyed in man or the mosquito—the ideal at which we should aim—then mosquitoes (larvæ) qua malaria may be disregarded I think the value of the papers of Lieut Col James and Lieut Col Gill lies in the fact that they have focussed attention on this—a somewhat neglected side of the problem It is reasonable to hope for considerable *advances by these means of malaria control* when more research has been devoted to them In the meantime however we can only desire for those engaged in anti larval work even greater success than they have already secured

Sir Malcolm Watson (F M S) replied Dr Natesan from Madras asked if I understand him aright what was to be done to control mosquitoes breeding in stagnant

the other *decrease the amount of infection* It may be argued that this policy will not enable an appreciable degree of 'control' to be achieved over malaria within a measureable period of time, but it may well be asked if there are any grounds for the belief that the age long association between man and the malaria parasite can be severed by any other means

The conclusion of the whole *matter is therefore that the final solution of* the malaria problem is still to seek and that it is inexpedient to rest satisfied with existing knowledge or with existing methods of 'control', but, whether the final *victory be the outcome of the slow biological method*, or the result of some startling discovery still hidden in the womb of time, let us not forget the advice of the immortal Harvey, 'to search out and study the secrets of Nature by way of experiment'

DISCUSSION

Dr A L Hoops (Straits Settlements) Anything emanating from the League of Nations has great influence in the Far East, and it is, therefore, with a feeling akin to dismay that workers in Malaya have read the League of Nations' Malaria Commission's recommendations for dealing with malaria in Europe

As regards Malaya we do not agree that the record of anti malarial campaigns is one of exaggerated expectations followed by disappointment and abandonment of the work We have at times made mistakes but we have learnt by our mistakes, and avoided them in other fields It seems to me that there can be no essential difference between the means to be adopted to reduce malaria in the island of Singapore and in the island of Corsica, *though there may be a difference in the amount of malaria and in the* Anopheline carriers in the two places We agree that the treatment of those infected with malaria and the destruction of the adult Anopheline mosquito in houses is *important, but we hold that the most important means of all is the control of Anopheline* breeding places which is the gospel of our great master, Sir Ronald Ross Bonification is good, and in addition we find that bonification in Malava greatly reduces the number of Anopheline mosquitoes (the Commission suggests that their numbers are often increased by efficient drainage and cultivation) But in our experience the healthiest labour force and the healthiest managers, living in well constructed lines and bungalows well fed and cared for, will go down in numbers if they are situated near a potent source of malarial infection

We cannot agree to that counsel of despair outlined by the Commission that we are not to try to eradicate the endemicity of malaria, but only to reduce the severity of the disease

We do not find that malaria becomes a disease of little importance when the sufferers are systematically treated with quinine Nor do we find, as suggested on page 22 of the Report that malaria can be cured in a few davs The picture shown on the screen by Col James of the habitations of the poorer parts of the population in Bulgaria, Russia, Italy etc, is astounding We have in Malaya our Saker aborigines whom the Malayees look on as very degraded *It would appear that the poor of Bulgaria are on* as low a plane How does Col James expect that such people will take a course of

quinine to cure malaria? How can their wives and children swat mosquitoes in the miserable hovels and troglodyte caves where they live? What would the cost of quininization of such a population be if it could be effected? I am of opinion and the figures given by Dr Scharff support me that the cost of permanent anti malarial drainage would be far less. I realize that this may be impossible in areas where the population is greatly scattered but surely there are many villages throughout Europe where anti malarial drainage can be adopted. In many instances this is a *very cheap* method where the breeding places are few and well defined. Near Port Dickson in the Federated Malaya States Javanese and Malaya peasant proprietors have themselves carried out anti malarial drainage at their own expense with successful results

In conclusion in Malaya despite the report of the League's Commission we will continue to pin our faith in the main to that very direct method of malaria prevention the anti larval which goes to the root of the whole matter

Major A Parker Hitchens (U S A) Considered that a part of the money given to Bulgaria should be definitely applied to anti malarial work in that country

Dr C Natesan Moodeliar (Madras) I have been listening to the papers read on anti malarial measures. The city of Madras which I represent here experiences certain difficulties. I have to place them before this Congress of the medical men from all over the world to have them cleared. Some years (about 15 years) ago there was an epidemic of malaria in a portion of the city. Almost every child in the locality had a large spleen. The place which was once a fashionable quarter for the well to-do to live in became deserted. Residents actually fled for their lives. Anti malarial operations were started. Wells pools and ponds were oiled and small fish were introduced into the wells. Most of the residents used well water for drinking purposes and they could not drink oiled water. Some of the residents were not fish eaters and they did not like fish introduced into these wells. Subsequently wells pools and ponds were ordered to be closed. Things returned to normal conditions. But the little patches of water in the city had disappeared so that when there was a drought this year the residents suffered for want of water. The Corporation resolved to dig up the wells. May I request this Congress to suggest measures for the destruction of the larvae beyond oiling and closing up of wells?

The residents complained that the epidemic might be due to the vicinity of the city sewage farm. Of course I believe that the water from the sewage farm percolated into the wells. The water in the wells was tinted yellow. It was said that rice cooked with it was also tinted yellow. May I request the experts who are here to let me know whether Anopheles can thrive in sewage water?

Last year the city of Madras had a severe mosquito pest which was unprecedented. Some years ago open drains were replaced by underground ones. Of course the latter were an improvement over the former but the mosquito nuisance was such that the residents were afraid of the approaching night. People suspected that the underground drainage was the cause especially the syphon connections. The executive of the Corporation proved to them that the syphon connections were not the cause. Anti malarial operations came into existence. Silt was removed from the underground drains about 500 lorry loads from one drain alone. The mosquito nuisance abated

THE SUCCESS OF A SCHEME BASED ON OUR SYSTEMATIC AND BIONOMIC KNOWLEDGE OF ANOPHELINES

BY

C STRICKLAND, M.A, B C,

Professor of Medical Entomology, School of Tropical Medicine and Hygiene, Calcutta

PRIOR to Ross's discovery there was quite a goodly array of items on the roster of anti malarial measures

Ross himself in his 'Prevention of Malaria' recites how the ancient Greeks and Romans viewed the matter He says 'the Greeks even at an early date had become aware that by drainage sickness could be avoided,' while 'the Italians have for a long time known how to control malaria by drainage and allied measures'

Coming to later times we read in Davidson's 'Hygiene and Diseases of Warm Climates' (Young J Pentland, 1893) that living near to marshy ground or the dry beds of summer torrents should be avoided, planting eucalyptus which dries up the soil should be encouraged subsoil drainage of towns put down, the neighbourhood of malarious indigenes eschewed, and so on, while Notter and Firth (in 1896) emphasize the importance of securing good drinking water and avoiding evil currents of air especially near to fœtid marshes It will be noted that while such measures were purely empirical, Ross's work did not invalidate some of them they were only placed on a more rational basis

The immediate consequence of Ross's work was to direct attention to the systematics and bionomics of the mosquito, and especially to the life of the larva as an aquatic creature, and this had the natural effect of re focussing anti malarial work largely on the drainage of marshes For instance Watson started on these lines in his early work (so soon in fact, as 1901) in Malay

But Ross's discovery had more far reaching consequences than this and the very terms of his announcement contained the seeds of further progress in that it was a dapple winged mosquito and no other that carried the parasite Hence the stimulus to the systematic study of the family which ensued

Ross narrates how in 1897 he could obtain no information in India—not even in the Indian Museum—about mosquitoes

But feverish activity was soon evident Ross himself started a careful study of his dapple winged mosquitoes and established their differential points from the other common sorts, and as the former only had been found susceptible to the development of *Plasmodium* he inferred that these observations would lead to economy in the practical prevention of the disease Theobald at home soon brought out his Culicidæ of the world while Giles' Gnats appeared in India (1902) In the latter however it is noticeable that not much progress had been made with regard to the larvæ However in 1903 Stephens and Christophers in their handbook had established the study of the larva and the bionomics and the relative importance of the species on a firm basis

The practical applicability of all this work was not lost on Watson, among others who by careful observations noticed that much anti malarial work depended entirely on the species of Anopheline present It was in those days something of a romance to find that the draining of a jungly swamp on the plains abolished malaria because *A umbrosus* would not live in the drains while to drain a swamp in the ravines of the hill land made matters worse because *A maculatus* preferred the drains to the swamp This discovery may be said to mark the turning point between what one may call the general way (it may be almost called the empirical way) and the specific way of dealing with malaria

The *general* way of dealing with malaria is exemplified by such measures as subsoil drainage earth filling training streams site selection for habitations prophylactic quinine etc These measures are general because they are equally effective whatever may be the species locally implicated in the incidence of the disease

The *specific* way of dealing with malaria is to ascertain the species which is locally responsible and to deal with it in its breeding places alone taking care that what one does is not a means of introducing another species which may be harmful Most anti malarial schemes to day are based on this procedure I have mentioned Watson s work in Malay and the Panama Canal Zone is another shining example

It will have been seen then in this short historical résumé that whatever anti malarial work has been conducted in recent years has depended on our systematic and bionomic knowledge of the mosquito

Now after the great amount of work though still insufficient which has been carried out it would scarcely be justifiable to narrate any account of another bit accomplished if it were not for the fact that it shows what can be done by a method not I believe hitherto deliberately put into practice It may have been tried in Malay but in India I think it has not, because when I suggested it for a scheme in Shillong the seat of the Assam Government in a place where it seemed to me to be eminently suitable I was informed that certain eminent malariologists in India did not believe in it I refer to a method based on the discovery in Malay that *maculatus* will not breed in jungle the method therefore being to let jungle grow over *maculatus* breeding places

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Ambutia Estate, Kurseong

The scene of the operations was at Ambutia tea estate below Kurseong on one of the spurs of the Himalayas facing the plains of Bengal (Plate XXI, fig 1)

The estate lies between about 2 000 and 4 000 feet above sea level, but some of it was in a sort of pocket of comparatively flat land composed of water borne detritus which was very porous and full of springs In the years after the war malaria had been severe *in 1918 the sickness rate being 63 per cent of the labour* force and in 1920 the death rate 45 per mille This making the administration of the estate difficult Dr Kingsley Ward who was in medical charge advised that a malaria survey should be carried out and Mr Webb the manager, assenting in May 1923 asked me to make one

The Malaria Survey

I found the spleen index 17 11 per cent not a very high one (but on one division it was nearly 50 per cent while on another it was nearly nil) but sufficient to be a serious matter to the very susceptible Paharia (or hill man) and his children In the stony nullahs swamps and in the estate drains *A maculatus* was found everywhere in numbers (Plate XXI, fig 2) and this was the only known malaria carrier found

Recommendations —The main recommendation made was to intercept by drains the ground water and springs feeding the swampy areas and thereafter to plant the local jungle vegetation in a riband over the drains so as to cover them up completely The banks of the drains in the light micaceous soil were very friable and when kept clean were always falling in, and the vegetation would have the additional advantage of supporting the banks of the drains

Executive work —Mr Webb the manager took up the proposals enthusiastically and has carried them through splendidly overcoming all the little technical difficulties which have arisen from time to time Plate XXI, figs 3 to 5 and Plate XXII figs 6 to 8 illustrate the work

Results —The results will now be recounted and in connection with them I wish to thank the doctor of the estate Dr Birendra Kumar Chakervertv, for the excellent records he has kept, not only since but before the operations

In the first place I am at liberty to say that Mr Webb feels that he would not care to be manager of the estate if it reverted to its condition before the work started

The malaria sickness rate is shown in Chart 1 It was 831 or 60 per cent in 1919 and this year 1927 allowing for an average in November and December it has been 158 or 9 per cent It is also shown in Appendix I

This is all the more satisfactory as a record large number (310) of new coolies has been recruited this year and the factor of non immune immigration enunciated by Christophers and Bentley (1909) has been operative If 310 new coolies can be imported with a sickness rate of 9 per cent, the results can be considered very satisfactory

CHART 1

As a matter of fact, not only in 1927, but in 1924 and subsequently, the recruiting rate has been steadily going up while the sickness rate has *pari passu* come down Table I shows this

TABLE I

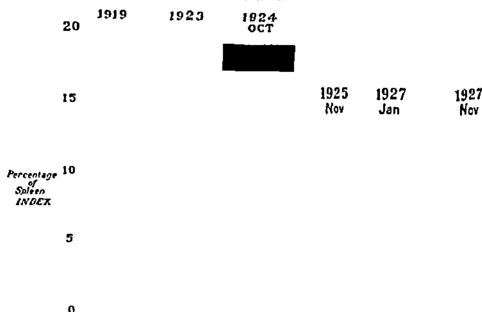
Year	Number of new coolies	Sickness rate per cent of population
1924	193	28
1925	242	18
1926	290	12
1927	310	3

The sudden rise in the sickness rate in 1924 over 1923 when it was 23 per cent must, I think be ascribed to the arrival of a large batch of new coolies before the scheme of operations had cost more than about Rs 600

The spleen index as is well known if the fever-rate is high the spleen index will also be high but the fever rate may be low when the spleen index is high

(i e, when malaria immunity is attained) The spleen index is therefore a safer guide to the endemicity of the disease in a locality On Ambutia, Chart 2 and Table II show the spleen index taken yearly since 1923 There was a slight

CHART 2



rise in 1924 owing no doubt to the increased sickness rate following the importation of new coolies mentioned above, but since then the decrease has been continuous I may add that Dr Chakervertv's results in 1927 showed a spleen index of 1 84 per cent (217 children) (i e, a trifle less than mine now) This November it was 2 per cent or 8 in 394 children We have the personal history of these 8 children as follows —

1 Lallbahadur Kami (Panchgharia lines), new to estate in 1926
2 Sinman Gimdar (Besseria lines) examined in 1925, 1926, Jan 1927, each time with negative results but now found with enlarged spleen
3. Dhwasay Chetri (Besseria lines) examined in 1925, negative, in 1926 ++ Jan 1927 + and Nov 1927 +
4 Kallay Jimdar (Besseria lines) examined in 1923 spleen +, 1925 + and Nov 1927 +
5 Maitay Damai (Besseria lines) 1925 +++, 1926 ++ and Nov 1927 +
6 Lakhu Limbooni (Besseria lines) 1925 ++, 1926 ++ and Nov 1927 +
7 Bikrama Newar (Besseria lines) new cooly last winter, suffered from malaria a lot this monsoon and found Nov 1927 +
8 Thutay Chetri (Tar lines) 1925 negative, Nov 1927 +

Some of these cases of splenomegaly may therefore be taken to be carry overs from pre-anti malarial operation days.

Other results —The *recruiting index* and *other vital* statistics I think any one with any experience of plantation labour will agree with me when I say that labour is extremely difficult to recruit on an unhealthy estate

Since 1921, the census year, we have reliable statistics of the population, and since 1924, of the newly-imported coolies (Table II)

TABLE II

Census year	Population	New labour	Remarks
1921	1,629		Big wastage of total population in spite of recruitment
1922	1,621		
1923	1,350		
1924	1,223	193	
1925	1,338	242	
1926	1,533	280	Recruitment compensates and more for normal wastage
1927*	1,769	310	

* Approximate

This table shows that not only has recruitment been assisted, but the wastage from death bolting, and other losses has been decreased considerably

General health —It is poor evidence but only those who saw the children both in 1923 and 1927 can realize the difference in their general appearance Now they are plump clear skinned bright eyed shiny haired, joyous little pegs of humanity, albeit not too clean Before they were wretched ragged, dirty, apathetic, tangle haired, and skin infected varmints Phthisis and hookworm are now the only two important endemic diseases on the estate and it is to be hoped that these will be reduced if only because of the improved malaria rate it is understood, moreover, that the directors are undertaking to house the labour under better conditions in future There is a good protected water supply and bowel diseases are not serious

Controls —I am afraid I cannot give any control evidence for our observations There is only one other tea estate in the neighbourhood and the agents inform me that malaria is not known on it

Summary of Results

To summarize the results on Ambutia one must conclude from a close analysis of the spleen index that there is still a small amount of endemic malaria on the estate

As a matter of fact what there is, seems to be now restricted to one division only (call it 'B') of the four on the estate The sickness rate and the spleen

indices on that division as compare l with the rest of the estate this year were as follows —

	B Division	*Rest of estate*
1927 sickness rate (*see* Appendix II)	20 per cent	4 6 per cent
November 1927 spleen index	4 80 per cent	0 7 per cent

Probably we may now say the rest of the estate is malaria free a big batch of new coolies would show how much remains

We have not yet found the source of the residual malaria in Division B The Balasun River flows below (*see* Plate XXII figs 9 and 10) at a vertical distance of about 1 500 feet and a gross distance of about three quarters of a mile

It is a prolific breeding ground for *A maculatus* and if at that distance it is a source of danger it will be difficult to deal with it

Cost —The cost of the work to date has been nearly *six thousand rupees* or about English £450 but that includes about a thousand rupees spent on oil etc before the scheme now reported on was started

CONCLUSION

I hope I may have persuaded you that we have had good results in consequence of our operations on Ambutia Estate and if so that the measure of deliberately planting ribands of jungle over *maculatus* breeding drains is essentially a practical proposition and a good example of the application of knowledge gained by research into the systematics and bionomics of mosquitoes

APPENDIX I

Year	Sick	Population	Percentage
1918	759	1 00 *	63
1919	831	1 400 *	60
19 0	75?	1 500 *	50
19?1	646	1 6?9	40
19	450	1 6?1	?8
19 3	315	1 350	?3
19 4	344	1 4	8
19 5	?39	1 339	18
19 6	188	1 534	12
19 7	158 †	1 763 partly estimated	9

* Approximate

† Including an average for November and December calculated from last quinquennium

Appendix II

Table showing comparison between Besseria and rest of estate

Year	Sick	Besseria only		Sick	Rest of estate	
		Population	Percentage		Population	Percentage
1918	243	330	73 6	516	870	59 1
1919	332	380	87 4	499	1 020	48 9
1920	345	400	86 25	407	1 100	37 0
1921	283	447	63 3	363	1 182	30 7
1922	138	440	31 4	312	1 181	26 4
1923	164	320	51 3	151	1 030	14 3
1924	113	236	48	231	988	23 4
1925	102	383	26 7	137	956	14 3
1926	58	425	13 6	130	1 109	11 7
1927	100 *	510 †	20	58 *	1 264 †	4 6

* Including an average for November and December calculated last quinquennium.
† Partly estimated.

EXPLANATION OF PLATE XXI

Fig 1 Showing Kurseong faintly on the crest of the hill 1,500 feet above, and the pocket of 'flat' land in front of the factory

„ 2 A streamlet now trained but formerly an extensive breeding ground of *maculatus*

„ 3 A ravine between bastis of tea land Drains overgrown with jungle now surround the ravine and the included area is planted with millet

„ 4 In the middle distance a ribband of jungle covering a nullah running down hill

„ 5 In the middle distance a mass of jungle growing in a ravine



Fig. 5

ON THE MALARIAL ENDEMIC IN THE CENTRAL PART OF JAPAN

BY

COL KATSUMI MATSUNO, I J A M C,
Professor of the Army Medical College, Tokio

I THE MALARIAL ENDEMIC IN CENTRAL JAPAN

THE malarial endemic in the central part of Japan is most prevalent in the vicinity of Lake Biwa and is rare in other districts Accordingly some of the physicians living in other districts where malaria does not occur are destitute of experience with this disease Tertian malaria is the only kind prevalent in Central Japan and the other kinds of malaria occasionally found there are the result of infection from outside Central Japan

In Central Japan new patients of malaria appear after the middle of June every year and the disease shows a rapid increase after the middle of July, but a marked decrease early in September A slight increase is again seen in the middle of September, but a rapid decrease towards the end of the same month, and there are almost no new cases in October The only species of the Anopheles found in Central Japan is, so far as I know, *Anopheles sinensis,* which begins to appear between the middle of May and early June of every year, and entirely disappears in October

Of the fifty or sixty cases of malarial patients which I examined among the troops in Japan proper from the latter part of autumn to the spring of the following year, every one had the history of previously suffering from this disease within the preceding ten months and many gametes could be demonstrated from the time of the onset of the disease Therefore, it would be no great error to consider all of them to be relapsed cases

Although it is a very difficult task to decide whether the new malarial patients who begin to appear from the middle of June every year are those who have been infected by the Anopheles mosquitoes which have survived the winter or by those which have newly emerged, yet we have the following facts —

(1) No larvæ of the Anopheles are found in the c [illegible] of Japan before the middle of May

(2) The eggs of the Anopheles mosquitoes laid in the latter part of autumn pass the winter in mud etc and become imagos under favourable conditions of temperature in the following spring

(3) Female Anopheles can pass the winter lying hidden in the straws or on the inner side of straw roofs But on examining 109 female Anopheles in the malarial district from December to April of the following year for the past ten consecutive years I could find no malarial parasites among them

(4) I bred 27 Anopheles mosquitoes making them bite and suck the blood of patients carrying many gametes of tertian malaria and making three of them once more bite and suck the blood of the patients I examined all of the 27 *Anopheles* mosquitoes during the months from October to December but found no imagos in them

From these facts it may be supposed that the malarial parasites in the body of the Anopheles are likely to die when the temperature falls and accordingly there may be no cases of malarial infection by the Anopheles mosquitoes which have just passed the winter

By various methods of provocation of parasites on the plasmodium *carrier* I found only gametes especially macrogametes in his peripheral blood If the gamete is the principal factor which causes the relapse of malaria why are there so many more relapsing patients in summer than in winter ? Also why do the cases in which no plasmodium could be demonstrated in winter relapse in summer ? It may be of course due to the fact that in summer there are many newly infected patients and the relapse may be caused by the stimulation of labour etc but it may also be due to the fact that there may be such a marvellous mechanism in living things that in winter the human body being free from the bite of mosquitoes the malarial parasites lie hidden deep in the body in a dormant state but appear again near the surface of the human body when mosquitoes appear in summer

I have observed 735 cases of malaria in the malarial district for the last twenty years The age and sex distribution of these cases is shown in Table I

From the above table we see that the number of patients are few in 1 to 5 *year* old infants and many in 6 to 15 year old children This may be due to the fact that infants are comparatively well protected against the bite of mosquitoes apart from the question whether they have congenital immunity against this disease In that district there is the custom of putting the infant under the mosquito net day and night in order to protect it against mosquitoes and flies The reason why the patients above the age of 21 years appear to rapidly decrease in number is because they do not apply for medical treatment as the symptoms of their *relapsing* fever become mild, or they become immunized The reason why in 21 *to* 30 *years* of age there are more female cases than male is because *many women* coming from the other non malarious districts in Japan to work *in sericulture* filature and tea-manufacture are infected by malaria

TABLE I

Age and sex distribution of the malarial patients treated at my res district for the last 20 years

Age	Number of Patients		
	Male	Female	Total
1—5	26	22	48
6—10	74	75	149
11—15	98	74	172
16—20	71	56	127
21—25	39	47	86
26—30	22	26	48
31—35	19	13	32
36—40	16	8	24
41—45	8	5	13
46—50	9	6	15
51 and upwards	14	7	21
Total	396	339	735

Of the 735 cases mentioned above, exclusive of 59 cases of which the dates of onset are unknown, 676 cases are shown in Table II, distributed by months which on the whole coincide with the monthly distribution of the cases among the I military troops for ten years, namely, from 1916 to 1925. T

TABLE II

Showing the monthly distribution of malarial cases treated at my residence for the last 20 years

Month	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	TOTAL
No of patients	15	13	14	11	13	86	152	147	97	58	28	26	676

TABLE III

Showing the monthly distribution of malarial cases among Japanese military troops for ten years from 1916 to 1925

Month	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	TOTAL
Troops in Japan proper	[illegible]	80	[illegible]	167	[illegible]	[illegible]	[illegible]	136	[illegible]	[illegible]	[illegible]	187	9 302 (including 1 death)
Troops in Formosa	[illegible]	[illegible]	[illegible]	[illegible]	416	[illegible]	[illegible]	776	633	[illegible]	840	505	6 596 (including 14 deaths)

As soldiers of the Formosan troops are very few compared with those of the troops at home the percentage of the patients among the Formosan troops is very great Especially the number of patients in winter is far greater among the Formosan troops than among the troops at home Among the troops at home the number of patients rapidly decreases in winter while among the Formosan troops the number of the cases only gradually decreases

In my several experiments concerning malarial infection in the human body I studied the mode of multiplying of parasites and the outbreak of the disease as follows I made 11 Anopheles mosquitoes suck the blood of the patient carrying many gametes of tertian malaria and 25 days after sucking I made the Anopheles bite myself and my three assistants At the end of 22 days there were found in one of my assistants 22 malarial parasites in 1 c mm of his peripheral blood but without any subjective symptom and afterwards the number of the malarial parasites gradually increased until the 24th day when the malarial parasites numbered 1 072 in 1 c mm of his peripheral blood and after nine hours the attack appeared

II METHOD OF TREATMENT

I tested various kinds of drugs especially for the treatment of tertian malaria and decided their efficacy according to the decrease in the number of malarial parasites found in the peripheral blood, their injury phenomenon, the influence on the symptoms, recurrence of the disease, etc But, whatever method of treatment may be used, the recurrence of the disease is usually unavoidable sooner or later, unless the after treatment is employed

(1) For the cases of tertian malaria and quartan malaria, administration of doses of 0 4 to 0 5 grm each of chin hydrochlor twice, 8 and 5 (or 4) hours before the attack, is recognized to be most effective, by which the chills are stopped in almost all cases In this treatment, schizonts disappear from the peripheral blood in 15 to 20 hours, and gametes in 25 to 35 hours, after administration of the first dose

(2) 0 5—1 0 grm of chin hydrochlor given at one time 5 to 6 hours before the attack is far less effective than the above treatment and no injury phenomenon appears in some schizonts

(3) Nocht's method of treatment is very convenient, but less effective than the first method

(4) Administration of a too small dose of chin hydrochlor seems rather to raise the resistance of the plasmodium

(5) The resistance of the malarial parasites against chin hydrochlor is generally weakest in macrogametes, and somewhat developed schizonts seem to have stronger resistance than those which are more developed, macrogametes being the strongest in resistance

(6) A dose of 0 4—0 7 grm of methylene blue given for a day at four or six different times is less effective compared with Nocht's method, but appears to act with comparatively great strength on young schizonts, especially on those young schizonts which have passed several hours after entering the red blood corpuscle

(7) Twelve c cs each of the blood serum, which was taken from a patient recently infected by malaria but not yet treated, was injected into a patient of tertian malaria during the apyrexial period and before the attack, but with no effect

(8) *Intravenous injection of neosalvarsan into the tertian malarial patient,* 0 15 grm during the apyrexial period and 0 3 grm five hours after the attack, does not show any remarkable effect in many cases But, if salvarsan is used when chin hydrochlor becomes less effective after continuous administration, the chin hydrochlor which is used afterwards will become fully effective

(9) I have observed that for the prevention of tertian malaria, it is most effective to give 0 4—0 5 grm of chin hydrochlor twice a day at intervals of four hours in the afternoon on every tenth day

(10) As the after treatment of tertian malaria, 0 4—0 5 grm of chin hydrochlor is given twice a day on every eighth day

OUTBREAKS OF MALARIA OCCURRING IN THE 'OFF SEASON'

BY

LIEUT COL W W CLEMESHA I M S (RETD)

Director Malaria Control Scheme Bandarawela

THE writer wishes to place on record some interesting facts concerning a certain type of outbreak of malaria which though perfectly well known to students of the subject are not fully appreciated at their proper value The subject to be discussed is outbreaks of malaria which occur in a season of the year when there is very reduced Anopheline prevalence when no active breeding is going on and when in the ordinary course of events the population is not suffering from the disease The occurrences described below took place amongst the labour force on tea estates they would have not been recognized at all had they been confined to the civil population The writer has been in charge of a large number of tea and rubber plantations during the past three or four years has carefully and fully investigated malarial conditions amongst many labour forces, has carried out preventive campaigns and met with some very remarkable successes

It is perhaps necessary to say a few words concerning the locality where the outbreaks occurred The district in question is a plateau about 3 000 feet above sea level in Travancore The climatic conditions in all malarial manifestations are of course an important factor At this altitude the nights are always cool even in the hot weather in the winter months of December, January and February the mean temperature for the month is under 70, largely due to the low minimum temperature at nights which is frequently as low as 50 the humidity when what is known as the 'land breeze' is blowing, is also low consequently during the months of December, January and February and sometimes also November the temperature and the humidity are so low in a normal year that no newly bred out Anopheles could elaborate a batch of sporozoites

The breeding places in this district can be divided into two classes —

1 The river Periyar itself situated at the bottom of the valley This river may be called a 'dead' river, in that the rainfall from some 200 square miles of catchment area is held up by the Periyar dam and the water diverted to another district The river below the dam now consists of a chain of pools over the rocks with a very small flow of water Occasionally in the heavy rains there is surplusing over the sill from the Periyar lake and occasionally the shutters of the dam are opened, when either of these occur, there is a certain amount of scouring

in the river itself but conditions that allow of this only occur in the monsoon period when it has very little influence on malaria

The valley of this river is intensely malarious, it is one of the very worst hyper endemic areas in Southern India The river breeds a large variety of different species of Anopheles but the important carrier is *A culicifacies* In some years this variety outnumbers all others by ten or twelve to one

2 The ravines on the hill sides Owing to the deadly nature of the valley, most of the lines occupied by the labour are placed well up the hill here there are innumerable springs patches of seepage and small streams, these used to produce large numbers of *A maculatus* and a few *A listoni* Many of the lines on the hill sides were nearly as malarious as those in the bottom of the valley owing to the prevalence of these species

The malarial prevalence of the valley presents the following features Anopheline breeding commences vigorously about the 1st of March In the winter months no larvæ can be found in the streams a few can be found at the edges of small swamps The writer has thoroughly investigated this condition and is confident that owing to the cold the minor breeding places in ravines on the hill sides do not produce any adult Anopheles during the winter months The output of adult Anopheles from the river is at this period very small indeed The importance of this will be seen later on The number of larvæ obtainable in breeding places on the hills increases rapidly during the whole of March In the Periyar river itself breeding is never active until the very end of the month but when once it has started the number of mosquitoes bred out is very large

Cases of malaria begin to appear among the labour force in the second or third week in April according to season A few of the early cases are infected with benign tertian but even at this stage malignant tertian is quite common During the second half of the month of April and the whole of May the number of re infections increases to an alarming extent by the end of May the whole labour force of say a thousand may be suffering from the disease as the crop of tea is usually heavy at this period the Company suffers enormous loss in consequence In the first or second week in June the monsoon appears from that time the health improves very rapidly the rainfall is from 100 inches to 150 inches 4 inches 6 inches and up to 10 inches in 24 hours are not uncommon as a result the breeding places in the ravines on the hill side are so scoured out that no larvæ remain The natural increase in the Anopheles of the area is absolutely cut short further heavy continuous rain for three to six days which is by no means uncommon certainly kills a large number of the existing female Anopheles in the neighbourhood of the lines By the end of June the health of the labour force is very much improved and from that time on cases that occur are usually relapses from the previous infective period As the cases are nearly all malignant tertian relapses are not as common during the next nine months as they are in localities where benign tertian and quartan are prevalent, this is notably the case in many districts n In a bad year it takes

three or four months of good diet and good medical treatment, commencing from the 1st of June, for a labour force to recover its good health and to be really productive, during the very worst of the epidemic many deaths may occur from a sort of nephritis due to the intensity of the malaria poison The above may be taken as a brief description of the locality and the normal course of events on a tea estate prior to the writer s arrival in the district Owing to very active anti malarial measures this depressing picture has now entirely changed, this, however, is not the point that it is desired to lay stress on in this paper

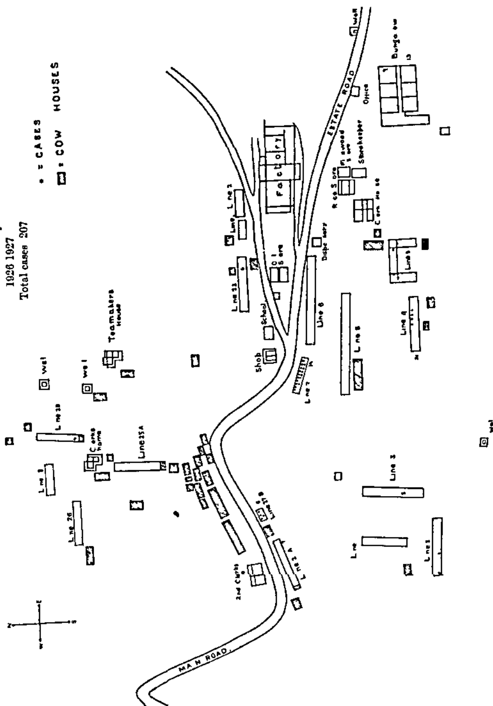
The writer was given charge of five very malarious estates belonging to a Company who possessed about twelve in this neighbourhood Those not put under his charge were practically free from the disease and it was not thought necessary to take skilled advice on their behalf In one of these so called non malarious estates known as 'Mount,' situated at an altitude of about 3 500 feet during the months of December January and February of 1926 and 1927 an outbreak of malaria occurred in two separate divisions on the estate which gave rise to 207 cases in a population of about 500 During the winter the writer was not present in the district which, of course, was very unfortunate, also the medical attendant on the estate said practically nothing about the occurrence until it was over but from very careful investigation made by the writer assisted by the group doctor there can be no doubt as to the genuineness of the outbreak and also that it was undoubtedly malaria spleen rates taken in the first week in March showed that 100 per cent of the children in one division and 60 per cent in the other were suffer ing from enlargement of the spleen On questioning the more intelligent coolies, they gave a perfectly clear account of their own attacks giving dates of onset, which exactly tallied with the record kept by the dispenser In order to save a lengthy description of what occurred a plan showing the general layout of the lines and the number of cases that occurred in each building, is also shown In this plan it will be observed that the most important feature disclosed is the very large number of cattle sheds in very close proximity to most of the lines To recapitulate, the main points of this outbreak were —

1 It occurred in December January and February, the three coldest months when Anopheles are very scarce and no breeding was going on in local breeding-places

2 The weather conditions would normally prevent the elaboration of a batch of sporozoites even if there was a constant stream of newly hatched females coming into the lines but there is some evidence that this year the climatic conditions were less severe and sporozoite formation may have been possible

3 There was no outbreak of malaria on this estate during the previous malarial season of March April and May of 1926

4 No anti malarial measures had been carried out in this estate and no particular attempt was made to reduce the reservoir of parasites amongst the children because it was not thought necessary, the estate being a healthy one


Malaria outbreak during
December January
and February
1926 1927
Total cases 207
= CASES
= COW HOUSES
ESTATE ROAD
MAIN ROAD
Factory
Office
Shopkeeper
Teamakers House
School
Shop
Line 6
Line 4
Line 3
Line 25A
Cattle home
2nd Clerks
Bungalow

5 In the five most malarious estates in the district no similar winter out break took place only a very few relapses occurring at this time of year on these Obviously therefore the outbreak on Mount was an isolated incident in the neighbourhood

From the above considerations it is practically certain that these cases were caused by infected female Anopheles who were passing the winter months in the cow houses but occasionally strayed into the lines and bit the coolies

The Anopheles was certainly *A culicifacies* This species has a great fondness for cow houses and is an important carrier in this district rivalling in importance *A maculatus* itself The writer has on several occasions captured hundreds of hibernating female *A culicifacies* in the middle of the cold weather in the thatch roof of a small cow shed about six feet by ten feet This prevalence of female *A culicifacies* in the cow sheds mentioned above is in all probability (the matter will be further investigated) an annual occurrence and why in this particular year there should be more infected females than normal or whether in this year alone sporozoite formation was possible is of course unknown The above is the only extensive outbreak that the writer has met with which occurred during the winter months but some isolated cases of great interest were brought to notice These will now be briefly referred to

During the same winter 1926 and 1927, a daughter of a neighbouring planter sickened with malignant malaria in Christmas week Dr J H Moore to whom I am indebted for these cases attended the case and spent one night on the estate His motor driver sickened with malignant malaria exactly ten days after this visit In the neighbourhood of the bungalow was a large cattle shed which normally contains 12 or 15 cows and calves As at Mount there was no local breeding at this period of the year and the climatic conditions were identical There can be no doubt whatever that these two cases were caused by infected female mosquitoes which were sheltering in the neighbourhood and probably in the cow house Investigation of the servants employed at this bungalow showed that the cook had a very large number of crescents in his blood though not actually suffering from fever at the time

As already pointed out the writer has been in close touch with malaria in this neighbourhood for three years and another point has struck him forcibly viz that in the very early weeks of March of each year odd cases of malaria occur frequently amongst European superintendents of estates One always has early information of cases of this nature and very careful investigation is possible In every instance parasites were found in the patient s blood It has been stated above that Anopheles breeding only commences about the first week in March in this district therefore it is quite impossible for that season s brood of Anopheles to be disseminating malaria in the first week in March Re infection cases which are due to the annual increase in the Anopheline population only begin to appear in second or third week of April therefore it follows that these very early cases (1st to 10th March) must be caused by infected females that have passed the winter

months in the neighbourhood and have become active again some time in February If this is not the correct explanation it may be pointed out that making the necessary allowance of ten days for the patient to incubate the disease ten days to *two weeks for the Anopheles to produce sporozoites* (which as we have shown would be impossible in most years owing to climatic conditions) and two to three weeks for the Anopheles to pass through the aquatic stage it follows that active breeding and laying of eggs was going on in the middle of January, which is practically impossible on account of the cold and is directly opposed to observations made over an extended period

The following are the cases alluded to above —

1 On two consecutive years 1926 and 1927 in the first week in March the superintendent of one estate went down with fever In both cases the parasites were malignant tertian and from the severity of the attacks they were very unlikely to be relapses

2 In the first week in March 1927 the steward of the Vandiperiyar Club had a very bad attack of malignant tertian malaria

3 *About the third or fourth of that month three or four planters gave a* farewell entertainment to one of their number who was proceeding home they stayed at the club till late in the evening Ten days later two of their number sickened on the same day with malignant tertian parasites in their blood they were living on an estate which was entirely free from cases of malaria at that time on which all breeding places were carefully oiled There can be very little doubt that they were infected at the club At that time of year the breeding in the Periyar river which was very close to the building had not yet started the river was under very careful observation by the writer at the time

4 In the first week in March 1926 the head clerk and the second clerk of a neighbouring estate both sickened within a few days of one another with malaria the parasites in this case were benign tertian All breeding places in the neighbourhood of the office were being oiled Prior to the starting of the oiling the writer went all over the breeding places himself and found that larvæ were practically non existent There can be little doubt that these two cases were infected by an infected female that has passed the winter somewhere in the neighbourhood very likely in the office itself

In the outbreak at Mount and the cases enumerated above the circumstances are identical The only explanation which satisfactorily explains these occurrences is that in this neighbourhood female Anopheles some of which were infected the previous year awoke to activity in the early spring (February) and before laying a batch of eggs succeeded in infecting certain number of human beings at a time when it is impossible for the new brood of Anopheles to have caused these cases This year on the estate under the writer s supervision special attention is to be paid to clearing *out the hibernating females in places where they are most* likely to be present

A FEW IMPRESSIONS ON A MALARIA SURVEY OF A GROUP OF TEA GARDENS IN ASSAM

BY

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ON 1st July, 1926 after a preliminary study of the various local species of Anopheline mosquitoes and their larvæ I began a malaria survey of the Labac Medical Practice

TOPOGRAPHY AND CLIMATOLOGY OF THE DISTRICT

The Labac Medical Practice is composed of eighteen tea gardens extending about seventeen miles in length by about seven miles in breadth and is situated in the Cachar District of Assam

The district of Cachar is a low lying plain broken up by isolated hillocks and natural depressions and surrounded by ranges of hills varying from 2 000 to 6 000 feet in height The plains are highly fertile and are interspersed with rice fields tea gardens clumps of jungle swamps rivers and streams The area surveyed although over two hundred miles from the sea is only about 70 feet above sea level

The climate is characterized by excessive humidity and is markedly oppressive during the monsoon season The hottest months are May to October with a mean temperature of about eighty three degrees the coldest month being January with a mean of about sixty five degrees The average rainfall is about 130 inches, being practically confined to the monsoon season during which period floods are liable to occur The plains form an alluvial tract—the constituents of the soil being clay sand and vegetable matter

DETAILS OF THE SURVEY

The survey began on 1st July 1926 and terminated on 30th June 1927 each tea garden being thoroughly examined on seven different occasions at intervals of about six weeks throughout the year

The survey of the breeding areas extended to about 1 000 yards from each group of coolie lines, and about a week was spent investigating each garden during each survey The maximum number of areas examined during one complete survey of the practice was 1 561

Excellent maps were provided by the managers in charge of the various gardens all breeding areas were carefully numbered, and a complete detailed record made of the findings in the numbered areas

In addition, adult mosquitoes were caught in human habitations and cowsheds throughout the practice to check the findings in the breeding areas and to study the feeding habits of the various species

Further a careful examination of all children between two and ten years of age who had been born and brought up on the respective gardens was made and the malaria spleen rate recorded The spleen rates in this district are not in my opinion vitiated by the possible complication of kala azar as the Labac Medical Practice appears free from the latter disease, apart from a very occasional imported case from the Sylhet district of the Surma Valley

Findings

From the statistics submitted, it will be seen that 166,738 Anopheline mosquitoes and their larvæ comprising eighteen species were examined and classified during the year

Of the total number classified 143 124 specimens were diagnosed in the larval stage 7 099 adults hatched out from larvæ and pupæ and 16 515 adult specimens were caught in nature

The eighteen species found during the survey with the percentage of each species were *A hyrcanus* 42 65 per cent *A fuliginosus* and *A philippinensis* 24 12 per cent *A aconitus* 8 08 per cent, *A vagus* 8 06 per cent, *A karwari* 6 20 per cent *A kochi* 4 03 per cent, *A barbirostris* 3 29 per cent *A minimus* 2 14 per cent, *A aitkenii*, 0 73 per cent, *A ramsayi*, 0 25 per cent, *A jeyporiensis* 0 17 per cent *A maculatus* 0 09 per cent, *A culicifacies*, 0 07 per cent *A leucosphyrus* 0 03 per cent, *A gigas*, 0 01 per cent, *A jamesii* 0 002 per cent and *A tessellatus* 0 002 per cent

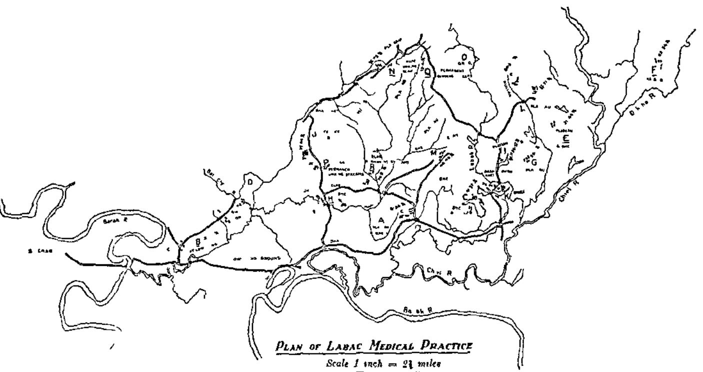
It will be seen that the two species *A philippinensis* and *A fuliginosus* have been grouped together under one percentage

The reason is that the diagnostic differences between these two species at any rate their larvæ and the larva of *A jamesii* have only recently been clearly defined by the researches of Dr Puri of the Central Malaria Bureau Kasauli

Likewise the erstwhile confusion which prevailed regarding *A jamesii* was cleared up by Major Covell Officer in charge of the Central Malaria Bureau Kasauli who honoured me by classifying a new species *A ramsayi* which had formerly been wrongly classified as *A jamesii* When our problems were elucidated we found that over 90 per cent of our so called *fuliginosus* group were actually *A philippinensis* and that *A jamesii* was a very rare species in this district

The distribution of the eighteen species throughout the practice shows that *A hyrcanus* *A philippinensis* *A aconitus* *A vagus* *A kochi* *A barbirostris* and *A minimus* were found on all the 18 gardens *A karwari* on 17 gardens *A fuliginosus* and *A jeyporiensis* on 15 gardens, *A aitkenii* on 11 gardens

MAP



PLAN OF LABAC MEDICAL PRACTICE

Scale 1 inch = 2¼ miles

A maculatus on 8 gardens *A leucosphyrus* on 7 gardens *A gigas* on 6 gardens *A tessellatus* on 4 gardens *A ramsayi* and *A jamesii* on 3 gardens and *A culicifacies* on 2 gardens With the exception of *A culicifacies* and *A gigas* adults of all the other species were caught in nature 13 565 were caught in cowsheds 1 657 in coolie houses 680 in garden hospitals 460 in babus *bashas* and 153 in bungalows

Specimens of all the sixteen species were caught in human habitations and all were caught in cowsheds except *A jamesii* and *A tessellatus* Only seven adult specimens of the latter two species combined were captured during the year The feeding habits as indicated by the relative percentage of each species (except the negligible number of specimens of *A jamesii* and *A tessellatus*) caught in human habitations and cowsheds show a preference for human blood only in the case of *A minimus A ramsayi A maculatus* and *A jeyporiensis* but perhaps the number collected of the last three species is rather limited to form a definite conclusion

The spleen rates in 3 465 garden born children from two to ten years of age on the respective gardens vary from 6 36 per cent to 76 81 per cent the average for the practice being 32 75 per cent An analysis of the causes of death for five years (19?? to 1926) shows that malaria was responsible for 16 42 per cent of the total death rate malaria convulsions being one of the chief causes of mortality amongst coolie children

Indirectly however by lowering resistance to intercurrent diseases malaria probably accounts for a higher mortality than the figure submitted

It is interesting to note that during the above period four adult coolies were admitted to hospital suffering from typical blackwater fever from which two succumbed

As a cause of sickness and chronic ill health malaria was responsible during the same period for 38 84 per cent of the total number of days under treatment of patients attending garden hospitals throughout the practice It should be remembered of course that malaria in the East like influenza in the West is the scrap heap for undiagnosed fevers The statistics also show that the malarial incidence is highest during the months of June July August September and October as compared with the remaining months of the year When sick rates and death rates are studied over a period of years many complicating factors such as *localized epidemics of cholera bacillary dysentery pneumonia measles whooping* cough febrile colds epidemic conjunctivitis Cachar sores (*Ulcus tropicum*) etc have to be eliminated before the effect of malaria on the health of a community as judged by spleen rates can be correlated on individual gardens Practical experience however teaches us that in gardens with high spleen rates there is a struggle for existence amongst children and unsalted imported recruits Those who survive undoubtedly acquire a modified immunity as has been shown by Christophers in his able researches on malaria in communities *living under* hyper endemic conditions

This salted element of a population living on hyper endemic malarious tea estates is indeed a valuable asset to vested interests otherwise in the absence of a modified immunity highly malarious tea gardens would rapidly cease to exist

Breeding Areas of Cachar Anopheles

A minimus —During the monsoon period breeds in clear grassy streams and drains especially where there is a certain amount of shade also in seepage from springs During the cold dry weather it is abundantly found in permanent rivers and streams in grassy tanks and swamps and in seepage water especially where wild saffron grows luxuriantly On one occasion it was found breeding in a small tank during the monsoon season We have not found this species in dense virgin jungle but it breeds freely in streams covered with secondary jungle

A maculatus —Breeds in clear running water in streams springs and drains exposed to full sunlight The edges were grassy in most of the streams in which *maculatus* was found breeding It was also found in seepage from springs A high percentage of sand in the soil appears to be a feature of *maculatus* areas

A ramsayi —Breeds in grassy tanks in permanent pools and swamps with clear standing water in which long grass grows abundantly

A hyrcanus —Breeds throughout the year in grassy pools rice fields tanks swamps borrow pits drains and at the edges of very slowly running grassy streams and ditches

A barbirostris —Breeds throughout the year in tanks and pools in which vegetation grows freely and at the edges of very slowly running streams shaded by jungle

A jeyporiensis —Breeds in clear running water in drains and streams in which grass retards the flow of the water

A fuliginosus —Breeds in seepage water tanks pools drains swamps and at the grassy edges of very slowly running streams

A philippinensis —Breeds in seepage water tanks pools drains ditches swamps, borrow pits rice fields and at the grassy edges of very slowly running streams

A kochi —Breeds in grassy pools and drains choked with vegetation It was also found on one occasion breeding in a *kutcha* well

A culicifacies —Was found breeding along the banks of the Cheeri river when at its lowest ebb during the month of March 1927 over two miles away from the nearest tea garden coolie lines Two larvæ were collected in temporary rain pools near coolie lines during the months of April and May

A gigas —Was found breeding in drains and streams which are only about 70 feet above sea level during the months of December January and February The breeding areas were at least twelve miles away from the North Cachar Hills.

A aconitus —Breeds in tanks with grassy banks in seepage water and in streams and drains throughout the year

A karwari.—Breeds in spring's seepage water in weedy tanks and pools, also in slow running streams and drains in which vegetation grows freely.

A leucosphyrus.—Breeds at the edges of slowly running streams and pools shaded with heavy forest jungle.

A vagus.—Breeds in stagnant water, in puddles, borrow pits and in rice fields.

A aitkeni.—Breeds at the edges of running streams and in pools and in water courses covered by heavy forest or secondary jungle and occasionally in tea garden main drains shaded by tea bushes.

A jamesii.—Only one pupa was collected during the survey. It was found in July 1926 in seepage water near the grassy edges of a very slowly running stream.

A tessellatus.—Four adult specimens were caught in nature during the months of April and May but the breeding areas were never located.

A FEW IMPRESSIONS

The importance of larval diagnosis in malaria survey work is evident when it is considered that 85 per cent of our total specimens examined and classified were diagnosed in the larval stage. If a survey depended entirely on the diagnosis of adults bred out from larvæ much important information would be lost owing to the high mortality amongst larvæ in collecting bottles. Further much valuable time and energy on the part of larva collectors would be wasted, apart from the additional expense involved in providing an enormous number of hatching bottles, increased laboratory accommodation and increased laboratory staff.

With regard to the breeding habits of Anopheline mosquitoes, certain species undoubtedly adhere to certain types of breeding areas. In a district, however, with a rainfall of over 100 inches which is practically confined to the monsoon season, new temporary streams and new collections of water of varying types make their appearance, their characteristics varying with the climatic conditions. The various species will then select breeding areas which they find most appropriate for the maintenance of their larvæ. Again when the cold and dry season comes round and the majority of the streams and drains become dried up, stream breeders such as *A minimus* will be abundantly found in permanent pools, abandoned tanks and in seepage water.

Under our marked seasonal variations in rainfall and climate it would appear to me that the data obtained from a survey of breeding areas limited to a few weeks (except possibly the months of October and November which combined include monsoon and dry season conditions) would be entirely unreliable in formulating anti-larval measures against a proved carrier species such as *A minimus*. A study of the breeding habits of *A minimus* shows that during the six months May to October it was found in 62 areas, whereas from the beginning of November to the end of April it was collected from 216 areas. During the latter period, when the residual water in streams, pools, tanks, etc., is at its minimum, there is a corresponding concentration of larvæ of practically all species.

As a practical point in anti larval measures the cold dry season would appear to be the most appropriate period to obtain the maximum effect with larvicides for owing to larval concentration in reduced surface water less larvicide is required to destroy the maximum number of larvæ and from an economic point of view fewer applications are necessary when the aquatic stages in the mosquito life cycle are prolonged by low temperatures The effect of flooding during the monsoon season as has been noted by Bentley is apparent Low lying gardens which are easily flooded have invariably low spleen rates whereas gardens on higher ground with running streams breeding *A minimus* are invariably highly malarious It is unfortunate that natural enemies of larvæ such as small surface feeding fish are found least in running streams where most required Our local species of fish—*Haplochilax panchax* (Kanpona) *Trichogaster fasciatus* (Khalse) *Anabas scandens* (Koi) *Nuria danrica* (Darkina) which feed on mosquito larvæ —abound in swamps, tanks and permanent pools to a lesser extent in low lying slowly running streams and drains but in these (their normal) habitats they appear to feed principally on the larvæ of harmless Anophelines The application of larvicides during the monsoon season should therefore be limited to the known breeding areas of proved dangerous carriers That *A minimus* is our most dangerous local carrier is being clearly demonstrated from the evidence which we are accumulating against it No breeding areas of *A minimus* could be found in the low lying gardens with a low malaria incidence and with spleen rates under 10 per cent during the steamy monsoon season whereas the malaria incidence and spleen rates on the other gardens varies with the presence prevalence and proximity of breeding areas of *A minimus* to groups of human habitations

Further the results of our dissections in an Anopheline Infectivity Survey partly financed by the Indian Research Fund Association which we are at present carrying out in this district clearly incriminates *A minimus*

With spleen rates varying from 6 36 per cent and 76 81 per cent in two gardens barely two miles apart with other instances in this Practice of a garden with a low spleen rate less than one mile away from a garden with a high spleen rate and above all with groups of coolie lines on the same garden separated only by a few hundred yards where spleen rates vary from under 20 per cent to over 60 per cent it is obvious that malaria is mainly a site infection This variation in spleen rates indicates when appropriate food and breeding areas are available the flight of certain species of Anopheline mosquitoes is very limited This opinion I also formed during 1918 when living on the desert at Kasr el Asrak in Transjordania

At Kasr el Asrak there are a number of pools in which Anopheline larvæ could be caught abundantly These pools are surrounded by vegetation As there were no other breeding areas and no human habitations within a radius of at least thirty miles the late Major W F Marshall M C R A M C and myself decided to investigate the flight of Anopheline mosquitoes from their breeding places We stayed at Kasr el Asrak for ten days and slept in the open air at night without mosquito nets along with a small party of Bedouin Arabs We found ' in the

stillness of the desert air sleep was impossible within 500 yards of the breeding pools from 500 to 1 000 yards the number of mosquitoes progressively diminished while over 1 000 yards we were unable to capture any specimens Certain species will however migrate for long distances as is evident when we consider that *A gigas* was found breeding in gardens over 12 miles from the nearest range of high hills during the months of December January and February *A gigas* apparently follows the climate and its appropriate breeding areas in the receding residual waters from the hills to the plains during the cold dry season

Apart from *A minimus* the only other species which we have so far found naturally infected with sporozoites in Cachar has been *A ramsayi*

This species is found on three gardens with spleen rates of 8 19 per cent 17 2 per cent and 23 13 per cent The species was prevalent only on low lying garden D which has a spleen rate of 17 2 per cent and here it seems to be mainly responsible for the malarial incidence In garden H, with a spleen rate of 23 13 per cent *A minimus* is also found to a mild extent during the monsoon season

The part played by other species such as *A maculatus A aconitus* and *A fuliginosus* etc stated to be natural carriers is being carefully investigated

A aconitus although it is one of the prevalent species in Cachar seems to prefer feeding on cow s blood and to date the few specimens which have been caught in human habitations and dissected have all given negative findings

A maculatus is regarded by Watson as being one of the chief carriers in Malaya We have found it in eight gardens but in very limited numbers The species appears to have a great struggle for existence in Assam during our marked seasonal variations in temperature and rainfall A more equable climate and a more evenly distributed rainfall perhaps accounts for the prevalence and importance of this species in the Federated Malaya States

A culicifacies although it is a well known carrier in India generally is so rare on the gardens surveyed that it probably plays a negligible part if any in Assam malarial incidence

We have still much to learn in Assam about malaria and mosquitoes and further research is essential before ill advised extensive expensive anti malarial schemes are embarked on With our present knowledge however much can be done to mitigate conditions in malarious districts

It is evident from our spleen rates and a knowledge of the breeding areas of a proved carrier species such as *A minimus* that site selection ' of coolie lines can greatly reduce malaria incidence Flooding as has been recommended by Bentley is nature s method in Cachar in the low lying districts during the monsoon season

Where site selection and flooding are not feasible a modification of Watson s methods viz open earth contour drainage converting drains and streams breeding *A minimus* into a series of still locks to make the areas less appropriate to the normal breeding habits of *A minimus* and to retain oil more efficiently has been carried out on gardens O and Q with excellent results during the past year The spleen rates on these two perennial highly malarious gardens have been reduced

from 60 27 per cent and 72 41 per cent to 35 93 per cent and 37 5 per cent respectively within twelve months

James in his recent laboratory researches states that malaria is mainly a house infection and this is indeed generally true in nature as we have found from our studies of the spleen rates and malaria incidence in individual human habitations I e Prince some years ago in the Panama Canal Zone advocated destroying Anopheles in houses Unfortunately this excellent method will only be carried out by the intelligent members of a community who realize the practical importance of this advice

Coolies can certainly be taught to recognize and collect Anopheline mosquitoes but my experience has been that monetary rewards for services rendered are essential and to demonstrate practically the success of their efforts coolies will invariably take the line of least resistance and collect their quota from cowsheds where harmless species abound and where carriers which have fed on human blood are unlikely to be found except when driven from human habitations by the smoke of cooking fires A recent suggestion by Deeks which is to be given a trial in the United Fruit Company s Plantations is to destroy Anopheles in human habitations by spraying insecticides

Screening of bungalows and hospitals and the provision of mosquito nets to coolies are all practical measures but general quinine prophylaxis after an extended trial on two of my highly infected gardens here in 1920 and 1921 was found to be disappointing and highly expensive

It is to be hoped that Plasmochin or a derivative of this preparation will be as effective in sterilizing gametocyte carriers as Salvarsan and its derivatives are in treponemal infections

The treatment of malarial splenomegaly by quinine and hæmatinics until more appropriate remedies are available is nevertheless essential

The importance of malaria prevention in Assam has lately come much into the limelight Malaria is undoubtedly the main medical problem in many tea gardens but it should be remembered that propaganda has its dangers for only a few years ago the pathological effects of hookworms were unduly stressed and resulted in many patients being surfeited with anthelmintics not only for chronic malarial cachexia but also for unrecognized post dysenteric anæmia and œdema or *Morbus benjalensis* a common clinical picture in Assam Further there are many tea gardens in this Province where the spleen rates and malaria incidence are low and where the perennial problems are not malaria ankylostomiasis or kala azar but the bacillary dysenteries the pneumonias and cholera

CONCLUSIONS

Eighteen species of Anopheline mosquitoes have been found in the Cachar District of Assam

The confusion which formerly existed regarding *A fuliginosus* and *A jamesii* has been cleared up In fact in Cachar both *A fuliginosus* and *A jamesii* are

comparatively rare as compared with the two species *A philippinensis* and *A ramsayi* with which they have respectively been previously confused

As anticipated by Christophers there is a close relationship between the species found in Assam and Malaya and probably when the Malayan Anophelines are studied *de novo* it will be found that the majority of the so called *A fuliginosus* species in that region should really be classified as *A philippinensis* *A minimus* which represents only 2 14 per cent of our total Anopheline findings is the chief carrier of malaria in Assam

A ramsayi representing only 0 25 per cent of our total findings was found breeding on three gardens and is a proved natural but apparently a mild carrier It appears to be mainly responsible for the malarial incidence in the garden with a spleen rate of 17 2 per cent

The part played by other species is still under investigation

There is need for further research in this Province but in the meantime much can be done with our present knowledge to mitigate the malaria incidence in malarious districts and here site selection of human habitations is of the greatest importance

A modification of Watson s anti larval measures has been carried out in gardens O and Q with apparently excellent results The practical measures which have been advocated by Bentley James Le Prince and Deeks can also be utilized to advantage where appropriate conditions present themselves

Finally if the malarial problem in Assam is to be efficiently tackled it is essential in my opinion to establish a provincial malaria bureau under Government control for unless expert guidance is locally available the efforts of district malaria boards as suggested by Sir Ronald Ross during his recent visit to Assam are liable to fail through want of skilled advice co operation and sustained action

Acknowledgments

I have to thank my establishment for the great assistance they have given me in this survey

Doctor Zillur Rahaman Chowdhury and Doctor Lalit Ranjan Dey were tireless and efficient workers in my laboratory

Assistant Surgeon C W Montgomery Doctor Prafulla Chandra Sorma Chowdhury and Doctor Girija Nath Chakravarty were employed on field work and did much excellent work under frequently very trying conditions

I am indebted to Col S R Christophers F R S and to Professor C Strickland for the great assistance they rendered to me during the initial stages of my survey to Major Covell and Dr Puri of the Central Malaria Bureau Kasauli for the prompt efficient and courteous manner in which they tackled our entomological problems whenever their help was solicited and to Mr J F Bagnall B SC Consulting Engineer Messrs Macneill & Co for kindly providing me with a scale map of the Labac Medical Practice

REFERENCES

COVELL, G (1927) A new species of Anopheles from Fastern India *A* (*My omy a*) *ramsayi* with a new description of *A* (*My-omy a*) *jamesi* (Theobald) *Ind Jour Med Res* Vol XIV No 4 April

Idem (19"7) A critical review of the data recorded regarding the transmiss on of malaria by the d fferent spec es of Anopheles with notes on distribution habits and breeding places *Ind Med Pes Memoirs,* No 7 July

CHRISTOPHERS S P (1924) The mecl an sm of immunity a_a nst malaria in commun t es living under hyper endemic cond tions *Ind Jour Med Res* Vol XII No 9 October

Idem (19"4) The Distribution of Mosquitoes in Pelation to tl e Zoogeograpl ical Areas of the Indian Fmpire Proceed ings 4th Fnt Meeting Pusa Govt Pr nting Press Calcutta

SEYMOUR SEWELL, R B and CHOWDHURI B L Indian Fish of Proved Util ty as Mosquito Destroyers

WATSON SIR MALCOLM (19 1) The prevent on of Malaria in the Federated Malay States

LE PRINCE J A and ORENSTEIN A J (1916) Mosquito Control in Panama C P I utman s Sons New York

JAMES S P and SHUTE P G (19"6) Report on the First Pesults of Laboratory Work on Malaria in England published by League of Nations Health Organization Malaria Commiss n Ceneva

DEEKS W F (1926) Fifteenth Annual Report Medical Department United Fru t Company General Offices Boston Massachusetts

ROSS SIR RONALD (1926 27) Malaria Control in Malaya and Assam A visit of Inspection 1926 "7 Poss Institute and Hospital for Tropical Diseases Putney Heath London

BENTLEY C A (1916) Note upon covering by water or flooding as a method of anti mal rial sanitat on Bengal Govt Pub

I URI I M (1927) Note on the full grown larvæ of *Anopheles jamesi* (Tl eobald) *A ful g nosus* (C les) *A pall dus* (Theobald) and *A ramsayi* (Covell) Cul cidæ D ptera *Ind Jour Med Res* Vol XV No 2 Oct ber

TABLE I

Total Anopheline mosquito findings (adults and larvæ) in the Labac Medical Practice from the 1st July 1926 to 30th June 1927

Name of Garden	A. hyrcanus	A. fuliginosus / A. philippinensis	A. aconitus	A. vagus	A. karwari	A. kochi	A. barbirostris	A. minimus	A. aitkeni	A. ramsayi	A. jeyporiensis	A. maculatus	A. culicifacies	A. leucosphyrus	A. gigas	A. jamesi	A. tessellatus	TOTAL
A	3 1[illegible]4	[illegible]	[illegible]17	1 660	[illegible]630	346	[illegible]08	91			11	1				1		10 516
B	4,256	1 449	413	50	48	[illegible]07	664	3		9								[illegible]6 3
C	3 437	2,891	115	1 [illegible]87	[illegible]84	448	139				1							8 6 4
D	9 361	3 3 9	206	180	31	71		3		[illegible]95	9			5				13 [illegible]6
E	5 8 3	1 677	331	7[illegible]0	38	159	465	[illegible]7	1					1	1			9[illegible] 3
F	3 099	[illegible] 547	119	1 430	7	[illegible]90	319	0	10								1	8 5 4
G	3 823	1 23[illegible]	9	1 [illegible]04	59	[illegible]3	57	67	1		3		130 [illegible] miles from coolie lines.				1	7[illegible]
H	8,548	4 551	574	516	[illegible]6	36	86	[illegible]3		43	6							14 409
I	5,865	1,558	1 8 [illegible]	4	486	[illegible]98	113	34			16							10 617
J	1 3 3	1 1[illegible]7	[illegible]1[illegible]0	38	34	53	1 09	1 047	36				1					6,921
K	9,183	7 9[illegible]3	1 100	2 8	[illegible]806	1 388	36	145			4	[illegible]		3	6			25 3[illegible]4

L	1 634	[illegible]	[illegible]	1 121	270	53	9	190			4						1	6 075
M	[illegible] 189	[illegible]	[illegible]	[illegible]	2,109	[illegible]	387	[illegible]	37		9	36		3	5	1	1	9 884
N	511	67	[illegible]	4	[illegible]	243	[illegible]	[illegible]	1 077		12	44		45				2,734
O	[illegible] 935	[illegible]	[illegible]	550	167	[illegible]	46	69	6		98	7		1	1			[illegible]
P	[illegible]	[illegible]	[illegible]	134		167	818	[illegible]	1		2	2						5 524
Q	812	590	380	393	813	689	527	103	18		107	61	1		5	2		4 501
R	2 183	810	[illegible]	164	[illegible]	590	[illegible]	[illegible]	12		12	1		1	1			7 577
TOTAL	1 114	[illegible]	13 498	13 455	10 342	6 734	5,494	3 575	[illegible]	417	[illegible]	153	132	59	19	4	4	168,738
Percentage	42·65	[illegible]	8·08	8·06	[illegible]	4·03	[illegible]	[illegible]	[illegible]	[illegible]	0·17	0·09	0·07	0·03	0·01	0·002	0·002	

TABLE II

Table showing the monthly findings of Anopheline mosquitoes examined in the Labac Medical Practice.

No.	Month and Year	*A. hyrcanus*	*A. fuliginosus* *A. philippinensis*	*A. aconitus*	*A. vagus*	*A. karwari*	*A. kochi*	*A. barbirostris*	*A. minimus*	*A. aitkeni*	*A. ramsayi*	*A. jeyporensis*	*A. maculatus*	*A. culicifacies*	*A. leucosphyrus*	*A. gigas*	*A. jamesi*	*A. tessellatus*	TOTAL
1	July 19[illegible]6	1 908	1 084	[illegible]5	[illegible]8	364	[illegible]9	188	27	10		49	43		33		2		4 000
2	August	3 934	4 272	42	136	1 848	87	100	6[illegible]	4		7			8				10 500
3	September	5 410	8 090	48	1 6 7	1 247	53	4[illegible]8	17										17 000
4	October "	[illegible] 808	5 7[illegible]6	96	4 595	947	574	359	127	16		9	39		4				15 300
5	November "	6 838	7 538	1 [illegible]90	2 618	1 [illegible]11	1 180	132	1[illegible]3	11	199	2	1		6				21 199
6	December "	5,[illegible]64	3 839	4 521	1 366	1 923	1 312	1 417	1 218	343		62	17		2	11	1		[illegible]1 405
7	January 1927	12 401	3 155	2 [illegible]94	742	393	1 000	830	4[illegible]0	[illegible]63	13	102			2	2			21 [illegible]06
8	February "	10,983	1,226	2,350	473	573	492	844	6[illegible]3	17	22	23			1	6			17 633
9	March	9 106	1 525	1 153	443	601	6[illegible]1	70[illegible]	[illegible]38	2[illegible]5	5	11	2	130 2 miles from coolie lines.					15 115
10	April "	3 [illegible]90	1 561	1 212	610	268	419	98	86	[illegible]	3	13	4	1	1			1	7 604
11	May "	4 0[illegible]0	835	335	435	341	[illegible]80	317	184	[illegible]5	6	8	34	1				3	7 414
12	June	5,012	1,382	42	26[illegible]	636	97	46	100	1	169	9	13		2		1		7 772
	TOTAL	71 114	40 [illegible]32	13,488	13 455	10 342	6 734	5,494	3 575	1 2[illegible]1	417	295	153	132	59	19	4	4	166 738

TABLE III.

Anopheline mosquitoes examined in the Labac Medical Practice from the 1st July, 1926 to 30th June, 1927.

Name of Garden	Mosquitoes diagnosed in their larval stage.															Total.
	A. hyrcanus.	*A. fuliginosus. A. philippinensis.*	*A. vagus.*	*A. kochi.*	*A. karwari.*	*A. aconitus.*	*A. minimus.*	*A. maculatus.*	*A. jeyporiensis.*	*A. barbirostris.*	*A. ramsayi.*	*A. aitkeni.*	*A. leucosphyrus.*	*A. culicifacies.*	*A. gigas.*	
A	7 068	2 080	1 629	370	2 544	216	83		11	201		2			.	10 106
B	4 106	1 316	495	109	43	400	3		2	632	79		.			7 205
C	3 350	2 717	1 273	440	200	114	21		1	132						8 314
D	8,814	3 066	155	67	27	202	2		9	19	232		5		.	12,608
E	5 618	1 329	701	131	32	342	14			384		1	1			8 733
F	2 749	2 316	1 243	200	1	119	680			304		10				7 640
G	4 761	1 123	1 120	15	81	87	30		3	56		1				6,783
H	8 011	4 709	435	30	18	530	21		6	86	43		100 2 miles from coolie lines.	.		13 859
I	5 070	1 309	390	279	463	1 811	34		16	100						10 071
J	1 490	965	16	31	4	2 098	1 009			1 069	36		1			6 534
K	5 633	3 673	1 113	289	1 470	944	120		4	19		2		4		13 316
L	1 503	2 373	398	50	208	122	169		2	28						5 558
M	2 873	1 588	310	093	1 646	169	156	31	8	515	34	3		2		8,312
N	222	—	1	16	16	12	61	21	6	213	1 059	35				1 691
O	2,771	2,653	453	290	111	573	56	1	87	34	6			1		7 027
P	1 006	636	179	156		1,612	312		1	610	21		.			5,383
Q	231	404	219	467	425	208	37	37	101	489	13			3		2,610
R	2 072	760	176	532	276	2 909	290		11	223	12	1		1		7 262
TOTAL	63,739	12 099	10 810	4 511	7 693	12,055	3,118	93	267	5,173	334	1,195	47	101	11	143,124

Anopheline mosquitoes examined in the Labac Medical Practice from the 1st July, 1926 to 30th June, 1927

Name of Garden.	Mosquitoes hatched out from pupæ and larvæ.																Total.
	A hyrcanus	*A fuliginosus* *A philippinensis*	*A vagus*	*A kochi*	*A karwari*	*A aconitus*	*A minimus*	*A maculatus*	*A jeyporiensis*	*A barbirostris*	*A jamesi*	*A ramsayi*	*A aitkeni*	*A leucosphyrus*	*A culicifacies*	*A gigas*	
A	67	60	11	19	49	1	5			5							217
B	88	106	7	5	[illegible]	13				10							[illegible]31
C	7	1[illegible]	14	8	17	1	1			7							[illegible]9
D	39	300	[illegible]5	4	1	4	1			3		36					66
E	107	135	44	15	3		4			68						1	377
F	188	[illegible]07	1[illegible]6	10			1			15							[illegible]08
G	146	96	8	7	5	5	31			1					30 2 miles from coolie lines.		403
H	[illegible]17	180	79	6		15	1										498
I	[illegible]36	185	31	19	[illegible]3	7				13							514
J	67	15[illegible]			1	[illegible]4	38			[illegible]							304
K	154	209	17	19	[illegible]4	31	8			4						2	468
L	53	[illegible]47	131		1	5	1[illegible]										449
M	161	9	10	155	101	4	16	2		41			3			3	588
N	11	11	9	5	13	6	8	11		8				3			83
O	129	[illegible]0	90	6	28	10	8	4	10	4							648
P	45	35		10		14	11			8							1[illegible]3
Q	69	44	[illegible]6	43	73	18	1[illegible]	5	5	[illegible]1	1		3		1	2	323
R	76	31	17	[illegible]3	2	43	7		1	5							[illegible]05
Total	2[illegible]8	2,6[illegible]1	69	354	343	201	175	[illegible]	18	235		30	6	3	3	8	7,099

TABLE V

The various places in which adult Anopheline mosquitoes were captured in the Labac Medical Practice from 1st July 1926 to 30th June 1927

No.	Where caught.	*A hyrcanus*	*A ful ginosus. A ph lippin nsis*	*A vagus*	*A kochi*	*A karwari.*	*A aconitus*	*A minimus*	*A maculatus*	*A jeyporiensis.*	*A barb rostris*	*A jamesii*	*A ramsayi.*	*A culicif.*	*A leucosphyrus*	*A tessellatus*	TOTAL.
1	*Coolie houses*	482	235	210	203	241	20	188	30	8	10	3	[illegible]0	1	2	4	1 657
2	Bungalows	0	42	30	6	4									1		183
3	Babus *bashas*	[illegible]13	170	25	19	17	10	4			2						460
4	Cowsheds	4 010	3 853	1 530	1 607	[illegible] 006	[illegible]95	55	8	2	68		7	19	5		13 565
5	Hospitals	30[illegible]	[illegible]12	4[illegible]	34	39	7	35		2	6				1		690
	TOTAL	5,077	4 612	1 837	1 869	2,307	332	282	38	12	88	3	27	[illegible]0	9	4	16,515

TABLE VI

Feeding habits of Cachar Anopheles.

No	Species of mosquito	Total number of Anopheline mosquitoes caught in cowsheds and human habitations.	Total number of Anopheline mosquitoes caught in human habitations.	Total number of Anopheline mosquitoes caught in cowsheds	Percentage of each species caught in human habitations	Percentage of each species caught in cowsheds
1	*A hyrcanus*	5,077	1,067	4,010	21 01	78 98
2	*A fuliginosus* }	4,612	659	3,953	14 28	85 71
3	*A philippinensis* }					
4	*A vagus* . ..	1,837	307	1,530	16 71	83 28
5	*A kochi* . ..	1,869	262	1,607	14 01	85 98
6	*A karwari*	2,307	301	2,006	13 04	86 95
7	*A aconitus*	332	37	295	11 14	88 85
8	*A minimus*	282	227	55	80 49	19 50
9	*A maculatus*	38	30	8	78 94	21 05
10	*A jeyporiensis*	12	10	2	83 33	16 66
11	*A barbirostris*	86	18	68	20 93	79 06
12	*A ramsayi*	27	20	7	74 07	25 92
13	*A aitkeni*	20	1	19	5 0	95 0
14	*A leucosphyrus*	9	4	5	44 44	55 55
15	*A jamesii*	3	3	..	..	..
16	*A tessellatus*	4	4	..	..	..
	TOTAL ..	16,515	2,950	13,565	..	..

TABLE VII

Showing Variation in the Number of Breeding Areas of A minimus during Monsoon and Dry Seasons

Name of Garden	Maximum number of areas examined on each garden	*Minimus* found in areas examined from 1st May to 31st October	*Minimus* found in areas examined from 1st November to 30th April.
A	8[illegible]		11
B	75		3
C	88		12
D	107	1 (early May)	1 (February)
E	85	2	7
F	67		11
G	120	3	11
H	116	4	4
I	78	1	14
J	60	1	33
K	110	2	11
L	93	8	18
M	1[illegible]	3	15
N	45	4	2
O	1[illegible]0	8	15
P	90	11	40
Q	46	6	9
I	67	8	[illegible]
TOTAL	1 561	6	[illegible]16

TABLE VIII.

Spleen-rates, Birth-rates, Death-rates and Sick rates in the Labac Medical Practice

Name of Garden	Spleen rate percentages in garden born children between 2 and 10 years of age in November and December 1926.	Average population from 1922—1926	Total population born and bred on the respective gardens, i e, salted population on 31st December 1926	Total births for five years 1922—1926	Total deaths for five years 1922—1926	Average annual birth rate 1922—1926	Average annual death rate 1922—1926	Daily average percentage sick on each garden from all causes during the five years 1922—1926
A ..	6·36	1,219	905	203	136	40·6	27·2	2·46
B ..	8·19	597	357	103	70	20·6	14·0	2·84
C ..	9·37	813	516	165	75	33·0	15·0	1·96
D ..	17·2	521	310	82	58	16·4	11·6	2·87
E ..	21·64	1,089	526	188	160	37·6	32·0	4·22
F ..	22·56	1,285	710	202	156	40·4	31·2	5·21
G ..	23·2	1,163	775	189	168	37·8	33·6	2·4
H ..	23·43	1,017	621	143	104	28·6	20·8	3·44
I ..	26·57	2,051	1,229	316	264	63·2	52·8	2·34
J ..	27·04	622	315	87	86	17·4	17·2	3·85

K	?8 64	1 183	8?6	1?9	123	35 8	25 6	3 12
L	33 71	1,593	1 142	347	235	69 4	47 0	4 01
M	46 46	994	408	182	166	36 4	33 2	2 71
N	56 73	455	287	69	74	13 6	14 8	5 93
O	60·27	1 407	728	233	231	46 6	46 2	5 89
P	67 27	582	298	94	80	18 8	16 0	3 09
Q	72 41	661	325	96	1?6	19 2	25 2	3 93
R	76 81	457	310	70	76	14 0	15 2	2 4
Total		17 713	10 618	2 947	2 393			

Total number of garden born children examined 3 46?
Total number of pos tive cases 1 135
Average spleen index for the Pract ce ? 7? per cent

Table IX.

Statistics showing average monthly incidence of malaria and other diseases in the Labac Medical Practice during five years 1922—1926

Month	Average number of days per mensem of patients under treatment for malaria (1922 1926)	Average number of days per mensem of patients under treatment for other diseases (1922 1926)	Total
January	5 201	8,665	13,866
February	4 356	7,910	12,275
March	4 885	9,240	14,125
April	5 315	10 893	16 208
May	7 297	13,427	20,724
June	9,179	15 855	25 034
July	10,365	15,198	25,563
August	10,240	13,727	23,967
September	8 561	11,316	19 877
October	8,104	11,774	19,878
November	7,120	10 170	17 290
December	7,019	9,812	16,831
Total	87 642	137,996	225,638
Percentage	38 84	61 15	

Table X

The chief causes of death amongst tea garden coolies in the Labac Medical Practice for five years (1922—1926)

No	Cause of death	Actual number of deaths	Percentage mortality of total number of deaths in the Practice
1	The Dysenteries (chiefly Bacillary)	552	23·06
2	The Pneumonias (Lobar and Lobular)	457	19·09
3	Malaria	393	16·42
4	Phthisis	258	10·78
5	Cholera	142	5·93
6	Nephritis	91	3·80
7	Blackwater Fever	2	0·08
8	All other causes	498	20·81
	Total	2 393	

MALARIA SURVEY OF PART OF THE LOWER BENGAL DELTA

BY

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This paper deals with the results of a recently concluded malaria survey of a portion of the Lower Bengal Delta in the neighbourhood of Calcutta Two hundred and ninety villages spread over an area of more than 300 square miles have been surveyed The country is a nearly flat alluvial tract and there is no fall of land

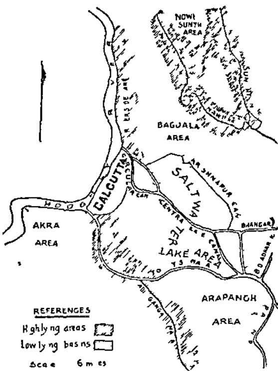


Fig 1 Map of area surveyed showing the different low lying basins and the high lying areas

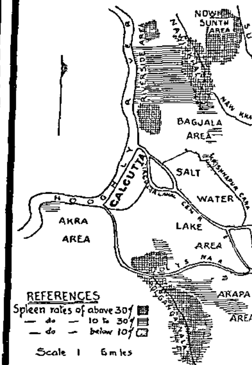


Fig 2 Map of the area surveyed showing the d of malaria in the different region

in any direction Here we have several large rivers which are tidal, like the Hooghly on the western border of the area, the Bidhyadhari on the east and the Piali on the south Besides these actively tidal rivers there are a few rivers which are tidal only in their lower reaches the upper reaches being outside tidal influence The Nawi and the Sunthi are examples of two such rivers The Adiganga once the bed of the Ganges is an example of a channel in a still more advanced stage of decay, as it is now too elevated to act as a water channel and is no longer reached by the tides But even in this essentially flat country there are some local elevations and depressions noticeable The banks of the tidal rivers form sets of twin ridges as a result of continual silting up of the banks until they have become the highest levels from which the ground slopes away Thus between two parallel rivers, are formed depressed areas which are natural basins in which rain water collects during the monsoons and from which there is no exit Thus the characteristic feature of the region now surveyed is an alternation of depressed natural basins with the elevated river banks The banks of the rivers Hooghly Nawi, Sunthi and the dead Adiganga form high ridges with depressions between them The Bagjala depression the Salt Water Lake depression and the Arapanch Auliapur depression are three such natural basins The area surveyed can therefore be divided into several natural divisions as follows —

A High lying areas
 (1) Adiganga area
 (2) Nawi and Sunthi area
 (3) Hooghly riverside

B Low lying areas
 (1) Bagjala area
 (2) Salt Water Lake area
 (3) Arapanch Auliapur area
 (4) Akra area

The entire area forms part of a flat delta This classification of the land into high lying and low lying areas does not imply that there are striking variations in the level of land as could be made out in an undulating or mountainous country But there is generally a difference of 15 to 20 feet between the levels of the high and the low regions and this small difference in level seems to account for the marked difference in the malariology of these areas as will be seen later

Nine species of Anopheles have been found here namely *Anopheles subpictus rossi*, *vagus culicifacies*, *fuliginosus pseudojamesi hyrcanus* var *nigerrimus barbirostris minimus* var *varuna* and *tessellatus* Of these *culicifacies* and *tessellatus* are somewhat rare while the other species are fairly common There is usually a large number of Anopheles breeding places to be found within and close to the villages here, such as tanks ponds drains ditches and marshes The rainfall is heavy, the annual average being over 60 inches of rain The greater part of the rainfall is confined to the months, June to September, the other months being comparatively dry

THE HIGH LYING AREAS.

Three areas are classed under this head, namely, the Adiganga, the Nawi Sunthi and the Hooghly riverside areas The characteristics of these elevated areas

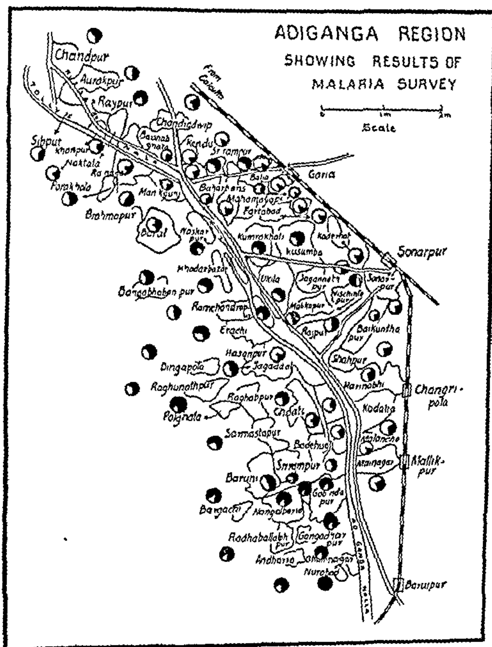


Fig 3 Map showing the spleen rates of the villages in the Adiganga area, one of the high lying dry regions here

are a general dryness of the villages the low level of the subsoil water and the sparsity of extensive collections of water The villages in the elevated areas are not subject to flooding during the rains as happens with the villages of the low lying regions There is also an abundance of vegetation chiefly of large trees dense shrubs and undergrowth which is usually absent in the low lying regions In the former area a village at a distance can only be denoted by a dense growth of trees and vegetation and the huts are scarcely discernible even from within the village, in low lying areas on the other hand the individual huts can be easily made out even from a distance

Adiganga Area—This area is situated on the elevated tract along the banks of the Ganges which at one time flowed over the land between Hastings and Barmpur Part of its bed is still marked by a series of depressions locally known as the Maraganga or the Dead Ganges This bed does not carry water at any time of the year The villages of this area are all dry and elevated even during the wet season there is not much stagnant water to be found in them At the close of the rainy season there is practically no water staying anywhere in the villages except in the larger tanks The entire belt of land from Chandpur on the north to Nurabad on the south is well wooded and the villages are densely overgrown with jungle In this area 48 villages have been surveyed and of these it was found that 4? villages had spleen rates of above 40 per cent nearly half the total number of villages had 60 per cent and above and 12 villages were above 80 per cent As many as seven villages were found to have spleen rates above 90 per cent and when it is found that most of the villages are of considerable size it proves beyond doubt that this is a hyper endemic zone Let us consider the spleen rates of the individual villages here starting with Chandpur on the north with a spleen rate of 55 we pass through villages having spleen rates as follows 47 65 33 43 81 70 74 77 71 55 70 84 61 89 98 100 and 96 The spleen rates seem to increase as we travel from north to south (Fig 3)

In this area there is usually a large prevalence of carrier Anophelines like *Anopheles varuna tessellatus* and *fuliginosus* and they are very common during the monsoon season At this time in every one of the villages a large number of small depressions like road side drains pits and ditches get filled in with the rain water and these temporary collections of water produce a large number of the carrier mosquitoes These breeding places disappear soon after the cessation of the *monsoons*

Nawi Sunthi Area—The next high lying area to be considered is the Nawi Sunthi area comprising adjacent groups of villages situated near the banks of the Nawi and Sunthi two rivers in a partial state of decay These villages are elevated and quite dry in summer there are generally very few collections of water to be found in them and even during the height of the rains there are not many noticeable large accumulations of water anywhere There is so much lack of water that a number of wells have been sunk in the villages here In such a dry region the

spleen rate has been found to be very high In the Nawi group 17 villages have been surveyed of which the lowest recorded spleen rate is 49 and the figure reaches as high as 94 per cent The majority of the villages have spleen rates ranging between 60 and 80 per cent with an average of 66 per cent In the Sunthi group of the villages ten villages were surveyed and of these, the spleen rates range between 50 and 85 per cent with an average of 58 per cent *It is clear therefore* that this area is another hyper endemic zone of malaria All the species of Anopheles previously mention d were found here but it was found that there is a large prevalence of *fuliginosus varuna* and *tessellatus* during the rainy season At that time there occurs a very disproportionate increase in the number of temporary breeding places in illustration of which a few instances are given here In Basirpara there are only five dry season breeding places while during the rains the number of temporary breeding places which hold water for more than one month numbered 58 Similarly in Gouripur the permanent water collections were 16 while the temporary ones were 75 It therefore happens that a survey of these villages conducted at any time other than during the monsoons would put it down that as the villages are quite dry, the number of breeding places are far too few Yet the villages are hyper endemic But the real state of affairs can be found if the survey is conducted during the rainy season when almost every small ditch or depression is filled in with water as the result of heavy rainfall during three months July to September a period which receives on the average about 35 inches of rain

These villages are very densely overgrown with vegetation consisting of large trees thick shrubs and dense bamboo clumps Even at midday the interior of some of these villages is quite dark and the midday sun is just able to send in a few rays here and there The prevalence of malaria in these villages is generally ascribed by the villagers to the presence of this dense jungle with the result that they believe that a clearing of the jungle would also clear the malaria of the locality

With this view a great many of the local efforts against malaria are directed chiefly to the clearing of jungle But in a country with a heavy rainfall a humid atmosphere throughout the year and a bright sun it is difficult to control vegetation in places which are most suitable for their growth The areas which are thus jungly are usually high lying dry regions with the subsoil water level low in such a soil and given a good rainfall this undergrowth flourishes very well and even within a few months of clearing at a heavy cost the place becomes over grown again *As a matter of fact* the presence of jungle in the deltaic area in Bengal is not the cause of the prevalence of malaria nor has it any influence over it as both are the products of other factors The presence of jungle in deltaic Bengal is however a good indication of the incidence of malaria usually in an endemic or hyper endemic state

Hooghly Riverside Area —Alongside the left bank of the Hooghly is a narrow strip of densely populated land running north and south covered by several large

municipalities and jute mills This is a raised ridge higher in level than the land to the east and the conditions here are similar to the other high lying regions discussed above especially the Adiganga area But the nature of the country presents an altered appearance as a result of a dense population There are many municipalities in this area and the entire river bank is occupied by garden houses and a large number of jute mills and quarters for the mill population

Here the spleen rates are very varying In densely populated mill areas it is usually lower than 10 per cent and it can be as low even as 2 per cent as in Khardah a highly congested mill area In municipal areas where the density of the population is not so great but is still much denser than the rural areas it ranges between 20 and 40 per cent In the typically rural areas of this elevated zone the spleen rates are usually above 40 per cent and may reach as high as 75 per cent There is no doubt that similar to other high lying areas discussed previously this is also a hyper endemic region judged from the nature of the country The area is dry elevated and in rural areas well wooded and jungly but it has been greatly modified from its characteristic endemicity by reason of the great industrial activity of the river bank where chiefly as the result of increase of population malaria has been greatly reduced The ordinary condition of such a region when not within a municipality or a mill area is to be seen in places like Rohra Patulia Mathpara and Napara the spleen rates of which are 41 77 50 and 73 respectively In these villages the houses are scattered the population is not dense and the nature of the country is typically rural During the rainy season a large number of breeding places come into existence in which carrier Anophelines like *varuna*, *fuliginosus* and *tessellatus* breed in large numbers In Napara there is a very large prevalence of *tessellatus* and *varuna* during the monsoons In Rohra out of 153 breeding places examined during the rains over a third of them were breeding *varuna* and this was found to be more prevalent than any other species at the time A similar high prevalence of *Anopheles varuna* was noticed in the village Patulia

If on the other hand we consider the mill areas like Sukchar Barrackpore and Khardah with spleen rates of 2 3 and 11 respectively the water collections even during the rainy season are few in proportion to the total population and even these collections of water are rendered unsuitable for the breeding of carrier Anophelines on account of the water being contaminated by various causes chiefly the inflow of sullage In such a case there is much *Culex* breeding but Anophelines with the exception of *Anopheles rossi* are very few

In the Hooghly river side area here discussed three different types are seen as the result of human activity The rural areas with a scattered population the municipal areas with a denser population and the industrial areas with great overcrowding exhibit different incidence of malarial endemicity The rural areas of this elevated region are similar to the Adiganga area in regard to their topography, breeding places and high spleen rates, while the municipal areas are less malarious and in the mill zone there is very little malaria

Congestion and overcrowding of population has resulted in a considerable reduction in the prevalence of malaria and the consequent lowering of the spleen rate In such areas there are fewer collections of water and as these are generally fouled chiefly by the inflow of sullage the breeding of carrier Anophelines is greatly restricted and this also contributes to the lowering of the spleen rates

The Low lying Areas

Of the low lying areas herein considered there are several The general characteristics of low lying areas are the excessive presence of water for a

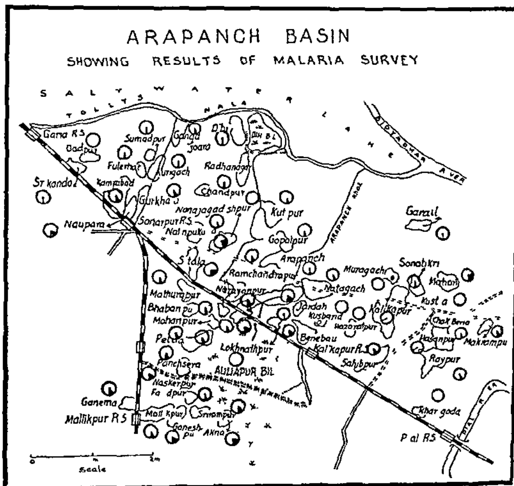


Fig 4 Map showing the spleen rates of the villages in the Arapanch basin one of the wet areas of the region

considerable period after the cessation of rains and nearness of the level of subsoil water to the ground level While in the high lying areas storm water does not stay very long in the low lying areas the breeding places have water for several months

after the cessation of the rains Further the proportion of temporary water collections to permanent collections is low Another characteristic of the low lying areas is the sparsity of vegetation Large trees are few and shrubby undergrowth is totally absent Elevated land in which the level of subsoil water is low is very favourable for the growth of rank vegetation while land which is low lying and in which the level of subsoil water is close to the ground is unsuited for the growth of this deep rooted undergrowth which requires a dry well aerated soil

Of the low lying areas surveyed here we have the following four —

(1) Auliapur Arapanch area
(2) Salt Water Lake area
(3) Bagjala area, and
(4) Akra area

The first three areas form parts of a continuous depression which have been separated since the construction of the Krishnapur canal on the north and Tolly's Nulla on the south

Auliapur Arapanch area is the large depression to the east of the Adiganga It is over ten miles long and five miles broad and extends from Tolly s Nulla on the north to the Piali on the south and is bounded on the east by the Bidhyadhari and on the west by the Adiganga area Being a low lying region it is subject to heavy flooding with storm water during the monsoons and since the edges are high there is no exit of water from this natural basin During the rainy season most of the villages in this area are flooded over on all sides with water and the people have to wade through water to get into their villages and in some places it is necessary for them to use small boats or dug outs In some of the villages the flooding is so heavy that only the centre of the village is above water Such flooding has been found to be very beneficial to the health of the village The spleen rates of all the villages here are consistently low out of 64 villages surveyed one half the number had spleen rates of 5 per cent and below and the average spleen rate for the entire area is 7 5 per cent This region in which there is much stagnation of water during the greater part of the year has very little malaria Here owing to excess of water the breeding of Anophelines is greatly restricted and the only species found breeding during the monsoon months in these villages are non carrier species like *Anopheles rossi* and *barbirostris* Here we find that during the malaria transmission season the prevalence of carrier species of Anopheles is very low

An interesting observation has been made in one of these flooded villages During the months July to October the ponds were breeding *A rossi* and with the close of the rainy season and the fall in the level of the flood water *Anopheles rossi* in the ponds is replaced by *A minimus* So, by the end of October and the beginning of November it is not unusual to find a large number of *A minimus* breeding in villages with very low spleen rates But this late season increase of a carrier species has no effect on the spleen index of the village which continues to be as before The mere presence of a carrier species is not enough, other factors like a high degree of their prevalence and the coincidence of their *numerical increase*

Congestion and overcrowding of population has resulted in a considerable reduction in the prevalence of malaria and the consequent lowering of the spleen rate In such areas, there are fewer collections of water and as these are generally fouled chiefly by the inflow of sullage, the breeding of carrier Anophelines is greatly restricted and this also contributes to the lowering of the spleen rates

The Low lying Areas

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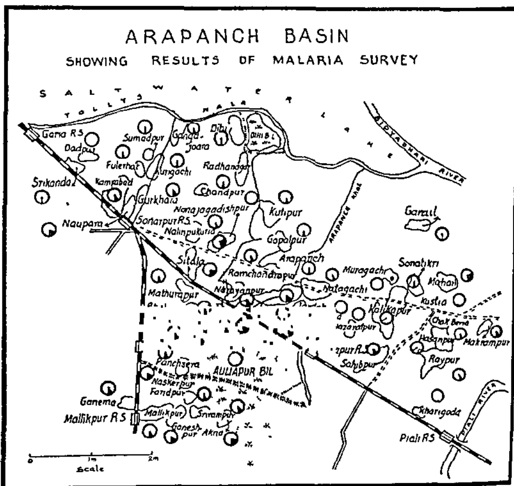


Fig. 4 Map showing the spleen rates of the villages in the Arapanch basin, one of the wet areas of the region

considerable period after the cessation of rains, and nearness of the level of subsoil water to the ground level While in the high lying areas, storm water does not stay very long, in the low lying areas the breeding-places have water for several months

after the cessation of the rains Further the proportion of temporary water collections to permanent collections is low Another characteristic of the low lying areas is the sparsity of vegetation Large trees are few and shrubby undergrowth is totally absent Elevated land in which the level of subsoil water is low is very favourable for the growth of rank vegetation while land which is low lying and in which the level of subsoil water is close to the ground is unsuited for the growth of this deep rooted undergrowth which requires a dry well aerated soil

Of the low lying areas surveyed here we have the following four —

(1) Auliapur Arapanch area
(2) Salt Water Lake area
(3) Bagjala area and
(4) Akra area

The first three areas form parts of a continuous depression which have been separated since the construction of the Krishnapur canal on the north and Tolly s Nulla on the south

Auliapur Arapanch area is the large depression to the east of the Adiganga It is over ten miles long and five miles broad and extends from Tolly s Nulla on the north to the Piali on the south and is bounded on the east by the Bidhyadhari and on the west by the Adiganga area Being a low lying region it is subject to heavy flooding with storm water during the monsoons and since the edges are high there is no exit of water from this natural basin During the rainy season most of the villages in this area are flooded over on all sides with water and the people have to wade through water to get into their villages and in some places it is necessary for them to use small boats or dug outs In some of the villages the flooding is so heavy that only the centre of the village is above water Such flooding has been found to be very beneficial to the health of the village The spleen rates of all the villages here are consistently low out of 54 villages surveyed one half the number had spleen rates of 5 per cent and below and the average spleen rate for the entire area is 7 5 per cent This region in which there is much stagnation of water during the greater part of the year has very little malaria Here owing to excess of water the breeding of Anophelines is greatly restricted and the only species found breeding during the monsoon months in these villages are non carrier species like *Anopheles rossi* and *barbirostris* Here we find that during the malaria transmission season the prevalence of carrier species of Anopheles is very low

An interesting observation has been made in one of these flooded villages During the months July to October the ponds were breeding *A rossi* and with the close of the rainy season and the fall in the level of the flood water *Anopheles rossi* in the ponds is replaced by *A minimus* So by the end of October and the beginning of November it is not unusual to find a large number of *A minimus* breeding in villages with very low spleen rates But this late season increase of a carrier species has no effect on the spleen index of the village which continues to be as before The mere presence of a carrier species is not enough other factors like a high degree of their prevalence and the coincidence of their numerical increase

with the transmission season are necessary for any increase in the prevalence of malaria

There is no doubt that in this area the people are greatly inconvenienced by the excess of water Whenever I went into the villages here the people invariably complained of the excess of water and remarked that it is necessary that the water should be drained away They do not appreciate that the low spleen rates of their villages is due to the excess of water which they are having It is therefore not surprising to find that in the Arapanch area a drainage scheme has been carried out to reduce the extent of flooding and to lower the level of water in the basin so as to bring under cultivation land which on account of its being too low was uncultivable The effect of this scheme for draining a deltaic low lying non malarious area on the health of the villages is being watched with great interest

Salt Water Lake Area —The Salt Water Lake is an extensive basin covering an area of 30 square miles and holding saline water which reaches a high degree of concentration during the summer months It is a large expanse of water interrupted here and there by a few villages and embanked footpaths In a region of this nature with water almost everywhere there are naturally few villages even though the area is extensive Even these villages are but sparsely populated and the inhabitants are chiefly fishermen who work the fisheries in the Salt Water Lake During the rainy season the level of water rises considerably in the lake and the villages then are isolated islands in the midst of a large expanse of salt water The only way to get to such villages is by the '*donga*' which is a dug out palm trunk In many of the villages in the Salt Water Lake the people have got to use these dug outs even for going from one house to another, and it is a common sight in some villages to find children swimming across the water to get to their neighbour s house

Within the villages there is barely any vegetation to be seen except one or two palm trees The vegetation of the lake area is a typically saline formation consisting of halophytes like *Acanthus ilicifolius* *Sueda maritima* and *Paspalum distichum* On the water are floating masses of algæ consisting of *Enteromorpha intestinalis* *Spirogyra* sp and *Oscillaria* sp

As the collections of water in these villages are always saline the inhabitants of this area have got to go outside the Salt Water Lake to procure their daily supplies of fresh water In many cases they have got to walk three to five miles to fetch fresh water During the rains when the footpaths are under water it is a common sight to see people punting homewards in dug outs and boats laden with pitchers of fresh water

In spite of the disadvantages the villages of this region are very healthy and the spleen rates are low Out of 27 villages surveyed 18 villages are below 5 per cent and several of them have a zero spleen rate The average spleen rate for the entire area is 6 per cent The common species of Anopheles found here is *Anopheles subpictus rossi* which breeds in enormous numbers in the brackish and

saline water In many of the villages it is the only Anopheline to be found The carrier species of Anopheles are comparatively rare in this area

Bagjala Area —The Bagjala depression is a continuation to the north of the Salt Water Lake depression but it has been separated from it since the construction of the Krishnapur canal This area is subject to extensive flooding during the monsoons and it is marshy practically throughout the year The Bengal Drainage Committee remarked at present the lower portions of this tract remain almost submerged during the rains and during high tides the water of the salt water marshes backs up and obstructs the drainage The whole area is unhealthy' On the other hand the results of the present survey show that the villages situated in this region are quite healthy the spleen rates are invariably low and the average for the area is only 5 per cent The common Anophelines here are *rossi*, *barbirostris* and *sinensis*

Akra Area —The Akra area is situated on the left bank of the Hooghly to the south west of Calcutta and unlike the other river banks this area is not elevated It is lower than the high tide level and it has been necessary to protect the land by means of an embankment alongside the river This area is traversed by a network of creeks and channels connected with the Hooghly which when these canals are open bring in and take back the tidal waters with every rise and fall of the river In this manner the more low lying portions of Akra area used to get the daily tidal flushings until recently when the important channels have been sluiced Twenty four villages have been surveyed in this area and the spleen rates vary between zero and 26 per cent The average for the entire area is 9 per cent which when compared to other areas similarly situated is a high figure The common Anophelines here are *barbirostris* and *sinensis* *fuliginosus* *varuna* *rossi* and *pseudojamesi* are also common

Summary

The survey covers a portion of the Lower Bengal Delta within tidal influence The area is flat but the banks of the rivers are usually elevated and between two adjoining rivers are depressed areas which form natural basins and which are subject to much accumulation of water during the monsoons The area is thus divided into different low lying and high lying regions

The high lying regions are usually dry well populated not subject to flooding during the rains and the level of subsoil water low The low lying regions are sparsely inhabited wet subject to flooding and water logging and the level of subsoil water is close to the ground level The three high lying regions here the Adiganga area the Nawi Sunthi area and the riverside area are hyper endemic zones The average spleen rate for the Adiganga area is 60 per cent and Nawi Sunthi area 61 In the rural riverside area the spleen rate is over 60 per cent On the other hand among the low lying areas the spleen rates are very low The average spleen rates for the Aubapur Arapanch area is 7 5 per cent Salt Water Lake area 6 per cent Bagjala area 5 per cent and Akra area 9 per cent There is thus a

very marked difference in the malaria prevalence of t
country The high lying dry regions are hyper endemic
regions have very little malaria (Figs 1 and 2)

The mosquito prevalence to a great extent brings
these different zones During the wet months of the
mission of malaria is greatest in the villages of the hi
numerous shallow ditches and drains which contain
carrier Anophelines to breed At that very time in
is heavy accumulation of water too great for the car
it has been found that at a time when in the high lyin
A varuna occurs in the low lying areas *this species*
The *varuna* breeding starts a few months later and l
increased to sufficient strength the transmission seas
month of November we survey a high lying village it
a few breeding places exist while a low lying village r
of the chief carrier species of this area *A varuna*
coincidence of the *increase of the carrier Anophelines* w
marks an endemic area while the non *coincidence* of t
malarious area

In the regions of the Lower Bengal Delta discusse
lying dry areas there is a coincidence of the two fact
in the low lying wet regions there is no such coincidenc
are non malarious The mere *presence or absence* of c
great material consequence This matter is now bein
in Bengal and a large number of villages are being k
monthly examinations of breeding places are being
variations in the periodicity of the Anophelines in diff

PLATE XXIII



Fig 1



Fig 4

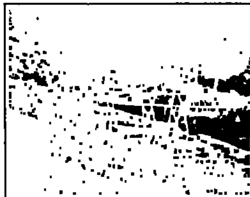


Fig 2

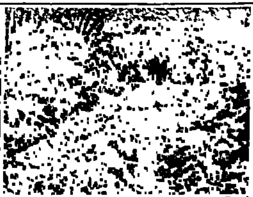


Fig 5



Fig 3



Fig 6

Explanation of Plate XXIII

Figs 1 and 2 Two views of the Nawi, a water channel in a moribund condition The stream runs through a high lying region

Fig 3 The interior of one of the 'dry' villages on the banks of the Nawi There is here an abundance of vegetation and the interior of some of the villages is dark even at midday

" 4 Another village in the Nawi area where the spleen rate is very high There are hardly any large collections of water to be seen anywhere and yet the village is highly malarious

" 5 A village in the Adiganga area, another endemic area The principal breeding places during the rains here are the small roadside drains which accumulate a little water

" 6 The interior of a village in the Adiganga area The photograph, which was taken at midday, shows how densely overgrown the villages are with vegetation There is great scarcity of water in these villages

Explanation of Plate XXIV

Fig 7. A general view of a portion of the Arapanch basin

" 8 Samadpur, a village in the Arapanch basin, which is surrounded on all sides with water during the rainy season Note the abundance of wet cultivation and the absence of dense undergrowth

" 9 Part of Fularhat village, in the Arapanch basin The spleen rate of this village is 3 per cent

" 10 The interior of Kurigachi village, also in the Arapanch basin There is barely any undergrowth to be seen in the village, but there are extensive collections of water

Plate XXIV



Fig 7



Fig. 8



Fig 9



Fig 10

(f) It should also be understood that the control work must be maintained continuously as any interruption in the work will cause a reappearance of many cases

JACOBO FAJARDO
Director of Health

APPROVED with the understanding that changes may be made from time to time as experience would indicate

F A GILMORE
Secretary of Public Instruction

Advisory Committee on Malaria—By an Executive Order an advisory committee on malaria control was created to advise the Secretary of Public Instruction The committee is composed of the Secretary of Public Instruction Chairman Director of Health, Adviser on Health and Sanitation to the Governor General, representative of the Rockefeller Foundation, a medical officer of the United States Army and the Chief of the Malaria Section as members and an officer of the Philippine Health Service as secretary

Personnel—November 1926 four physicians and three technicians 1st January 1927 field staff of the Rockefeller Foundation was transferred to the section September 1927, the staff is composed of eight physicians four technicians one entomologist, two field directors two assistant field directors one clerk 25 control labourers and one chauffeur Some 90 labourers who spray the *Paris green are paid by the localities under control The personnel* is organized into five control units each consisting of one doctor one field director (preferably an engineer) one assistant field director, one technician and five control labourers The duties as recommended by Mr J J Mieldazis of the Rockefeller Foundation are as follows —

Physician —
- (a) In charge of Unit personnel
- (b) Responsible for control work
- (c) Gather statistics
  - (1) Determine malaria incidence
  - (2) Collect blood films
  - (3) Make spleen indices
- (d) Report progress of work and endeavour to extend activities

Field Director and Assistant Field Director —
- (a) Make sketches of proposed control areas
- (b) Make mosquito surveys
- (c) Prepare working programmes for field labourers
- (d) Submit estimate of cost
- (e) Direct control operations

Technician —
- (a) Examine blood films
- (b) Identify mosquito larvæ
- (c) Dissect adult mosquitoes

Control Labourer —

(a) Supervise and direct operation of field labourers in malaria control

(b) Make house to house canvass each month and report the number of malaria cases

(c) Make and report systematic inspection for Anopheles mosquito larvæ and adult mosquitoes

(d) Submit weekly report of progress to the physician in charge

Distribution of Units —The Units are distributed as follows —

Unit I —Calauan Laguna Ten control areas Control started by the International Health Board, October 1926, plantations and two towns

Unit II —San Jose, Mindor Ten control areas sugar plantations Control started January 1927

Unit III – Kulambugan, Lanao Ten control areas Lumber concessions Control started August 1927 Previous to this time this Unit was at large in Mindanao making surveys

Unit IV —Novaliches Water Project Eight control areas Control started early part of 1926

Unit V —Bayombong Nueva Viscaya Six proposed control areas Newly organized unit controlling towns and barrios Control started September 1927

Previous Work on Malaria —Since 1909 streams were associated with the disease by Nichols In 1914 and 1915 Barber and Walker incriminated *A febrifer* (*minimus*) and to a less extent *A maculatus* as the transmitters of malaria in the Philippines In 1924 and 1925 the Rockefeller Foundation representatives incriminated *A minimus* and *A ludlowi* stream and river breeders respectively Barber and the latter workers suggested the use of larvicides as an economic means of control

Statistical Data on Malaria —Official statistics are unreliable and over exaggerated (21,267 malaria deaths out of 197 779 total deaths in the provinces in 1925) The reason for this is due to lack of study laboratory facilities and medical attendance and consequently 80 per cent to 90 per cent or more of the death certificates were signed by non medical men (the municipal secretaries) Wrong conception among medical and non medical men of the origin of malaria from swamps marshes mangroves, etc and the use of malaria as a scapegoat ' of diagnosis Examples A province which reports annually 1 200 deaths from malaria on survey and re survey by two members of the section at two different times showed no splenomegaly nor blood parasites in the children in four of the districts reporting the highest malaria deaths In an agricultural colony, 138 people lived for two years and all suffered many attacks of malaria, without a single death from any cause Children here had 90 per cent splenic enlargement and 54 per cent blood parasites about 50 per cent of which were crescents

Surveys —The results of the surveys in 20 provinces and Manila are shown in Table I Most of the surveys are on school children below 15 years as they are more permanent Spleens are palpated with subjects standing Out of 105

TABLE I

Province	Area Sq M	Population	No places surveyed	No with malaria.	Spleen Indices	Average	Blood Indices	Average	Predominant Species
Laguna	6?9	195 546	19	19	0-77·42	9 4	0-22	3 15	A A
Mindoro	4 0?4	71 931	14	14	13 8-75	28 60	0-25	5 52	Tertian
Pangasinan	1,193	565,7?2	21	9	0- 8 5	1 7	0-16 6	1 37	Tertian
Zambales	2 1?5	83 750	5	5	0-27 88	11 05	8-12 7	4 70	A A
Tayabas	5 993	212 017	2	1	0-17 5	14 84	0-9 7	8 51	Tertian
Davao	9 ?07	108 222	8	6	1 6-89	32 93	0-11 8	4 26	Tertian
Pampanga	868	?57 6?0	8	7	0-5 4	? 55	0-3 8	1 43	A A
Bulacan	1 173	249 ?92	4	3	0-3 07	1 61	0-3 5	1 45	A A
Lanao	? 000	91 459	10	8	0-45 4	23 24	0-15 9	9 03	Tertian
Agusan	4 ?04	44 740	9	3	0-55 0	12 60	0-8 0	2 47	Tertian—A A
Misamis	3 77?	198 943	4	0	0-1 0	0 .8	0-0	0-0	
Sulu	139	172,??6	9	6	0-90 00	11 62	0-54 5	7 34	Tertian

Cotabato	11 786	171 978	5	2	0–68 3	51 48	0 22 0	6 12	Tertian
Rizal	733	230,256	7	4	0–84 0	3 77	0–53 0	3 54	Tertian
Zamboanga	3 056	143 333	12	5	0–46 0	8 40	0– 9 9	2 69	Tertian
Bataan	537	58 340	13	2	0–3 24	0 73	0 1 33	0 14	Tertian
Manila	14	312 000	1	0	1 5 prisons	1 5 prisons	044	044	Tertian—Quartan
N Ecija	2 109	227 006	6	6	6 41	18 43	0 22 6	9 6	Tertian
Batangas	1 201	340 199	2	-	31 6 (Look)	31 6	23 3 (Look)	23 3	Tertian
N Vizcaya	1 9 0	35 838	10	10	7 59—45 7	17 57	5–29 8	17 00	Tertian
Bukidnon	3 871	48 544	4	3	26 8—43 0	2 52	None taken		
TOTALS	61 239	3 819 602	173	115					

Total persons examined				
	Spleen	18 151	Positive	1 321
	Smears	19 303	Positive	787

surveys the spleen indices were higher than the blood in 60 about equal in 21 and lower than blood in 24 Blood smears are prepared and examined according to Barber's thick smear method The type of the parasite is identified in the thin smear when necessary Tertian is the predominant parasite

Topography and Malaria —Malarious districts are always associated with streams or grassy irrigation ditches except two places not yet thoroughly studied Seventy seven per cent of the malarious districts are in the mountains or hills or very near them Eighteen per cent in inland plains and the remainder coastal or lakeside

Rain and Malaria —When rain causes flooding of permanent streams malaria subsides When rain causes the formation of new streams in localities where there are no permanent streams malaria appears Both conditions may be present in the same locality, hence two malaria seasons

Rice growing Region and Malaria —Unless near mountains or hills rice growing regions are not malarious

Anopheles Surveys —The article by F Banks gives the species and larval descriptions of those so far found in the Philippines Dissection of stomach and salivary glands of wild catches is in progress So far only one salivary gland and two stomachs have been found infected in 1 085 *A minimus* and none in other species The species and number dissected is as follows —

Anopheles funestus (minimus)	1 085
vagus (*rossii* pool type)	26
barbirostris	34
hyrcanus	51
, *karwari*	17
maculatus	1
fuliginosus	97
philippinensis	5
, *tesselatus*	2
TOTAL	1,318

Species Control —In Mindanao survey it was found that when *A minimus* was absent other species present, there was invariably no malaria When there was malaria *A minimus* was always found either alone or with other species Upon this 'species control' or 'species sanitation' was adopted controlling only streams and *minimus* breeding irrigation ditches In 69 malarious districts in Luzon and Mindoro *A minimus* was present in 64 or 93 per cent and the predominant species in 53 or 77 per cent If larval collections were done during the malaria seasons in all places *A minimus* will probably be the predominant species in almost all these places

Administration of Control —Del Carmen Plan A control unit (see personnel) supervises ten control areas An area is one with a radius of 1½ kilometres from the centre of the residential district Population of an area is from a few hundred to a thousand or more people Philippine Health Service Plan Control to be supervised by the regular sanitation personnel of the Philippine Health Service in the provinces Each plan has about 50 control areas each

Method of Control —All *minimus* breeding in a control area are sprayed once a week, except during heavy rains and floods with a one per cent mixture of Paris green in dry fine road dust Paris green should have at least 50 per cent of arsenious oxide The road dust is passed through a 60 mesh wire screen

Cost of Control —Del Carmen Plan The average operation cost per unit is p 17,120 per annum or p 1,712 00 per area, excluding quinine The *per capita* cost is from p 2 75 to p 11 50 The Paris green is sprayed by means of a hand blower Under this plan the number of control areas is limited

The Philippine Health Service Unit is made up of a travelling physician and technician whose total salary is p 2 600 per annum exclusive of travelling expenses This unit surveys, gives field instructions on control, and makes the re surveys The supervision of the labourer who sprays the Paris green is done by the sanitary inspector of the locality Paris green is sprayed by hand In this plan control becomes permanent as it becomes a part of the regular duty of the local sanitary inspectors The actual cost would be Fifty to eighty pesos per annum for Paris green and the wages of the labourer (about p 360 per annum) for each area about four times cheaper than the Del Carmen Plan Under the Philippine Health Service Plan, control can be extended to any number of areas

Results of Control —Will be known after re surveys which will be started about January 1928 Table II shows the results of control in two places —

TABLE II

Place	Blood Indices			
	1924	1925	1926	1927
Florida Blanca	17 8	3 4	0 5	1 1
Porac	12 9	4 1	0 3	1 2

MALARIA GENERAL

HABITS OF ANOPHELES IN RELATION TO THEIR ROLE IN THE SPREAD OF MALARIA

Importance of Monthly Differences in the Length of Life of *A maculipennis*

BY

Lieut Col S P JAMES M D, I M S (Retd),

British Ministry of Health, London

W D NICOL

AND

P G SHUTE

I propose to draw attention to one of the results which emerge from the arrangements which exist in England for providing supplies of infected mosquitoes to be used for inducing an attack of malaria in patients suffering from *certain mental diseases* At the British Ministry of Health we began to prepare batches of infective mosquitoes for that purpose in December 1923 and, except for an interval of about five months in 1924, we have prepared one or more infective batches each month since that time Up to October 1927 we have prepared 41 batches During this period of more than 3½ years we have not found it necessary to vary our routine technique for preparing infective mosquitoes To begin the preparation of a batch we collect about 300 or more specimens of *maculipennis* in the adult stage and we feed them upon a suitable case daily and incubate them at 23°C until they become infective During this procedure which occupies roughly a fortnight a large number of the mosquitoes die If we begin with 300 mosquitoes it often happens that only 100 or less will be available on the date when sporozoites are first present in the salivary glands The following is a statement of the numbers of mosquitoes used during each of the different months comprised in a period of more than three years and the numbers (and percentages) which survived until they became infective —

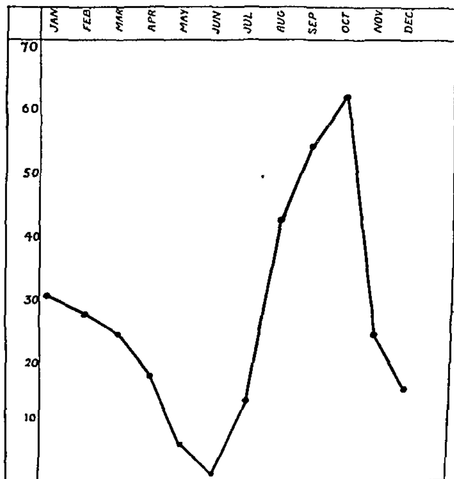
	Number of mosquitoes at the beginning of the procedure	Number which survived to become infective	Percentage which survived to become infective
January	390	116	30
February	130	35	27
March	665	162	24
April	550	96	17
May	334	21	6
June	1 298	213	1.7
July	1 800	239	13
August	1 700	724	42.5
September	500	265	53
October	330	220	66
November	740	176	23
December	300	47	15

It can be seen at once that many more mosquitoes die in some months than in others If we try to prepare an infective batch in June, less than 2 per cent of all the mosquitoes with which we begin the batch will be alive when the batch becomes infective, but if we prepare a batch towards the end of August or in September or October at least 50 per cent of the mosquitoes with which we begin the batch will be available for use in infecting patients The percentage of survivals falls again in November, and, after remaining at about the same level until the end of March drops suddenly in May and June and begins to rise again in July The diagram (Fig 1) illustrates the phenomenon

Before considering the significance of this diagram in relation to the spread of malaria I must refer to the probable cause of the high death rate of our mosquitoes in some months (particularly May to July) and the low death rates in other months (particularly August to October) We think that the cause has to do with growth and maturation of the eggs and with oviposition If one collects adult female *maculipennis* in England in May and June one finds that the ovaries are well developed in nearly all and that in a very few days the eggs become ripe and must be deposited The period is one of great peril to the mosquito's life and not many of them survive it In those that succeed in living through this critical time a second batch of eggs begins to develop almost at once, with the result that within a few days the insect has to go through a second dangerous experience of the same kind, to be followed (in the rare event of survival) by a third

Undoubtedly this is the chief cause of the very low survival rates of *maculipennis* during the early summer months in England

Now towards the end of August the findings rather suddenly change One no longer finds that the majority of adult female *maculipennis* caught in nature contain developing ova *There is an almost complete cessation in the growth and*



MONTHLY PERCENTAGE OF MOSQUITOES WHICH LIVED LONG ENOUGH TO BECOME TRANSMITTERS OF MALARIA

Fig 1

maturation of the eggs, a cessation which seems to be independent of atmospheric temperature—for when these insects are fed upon cases and incubated as usual, no increase in the size of the ovaries is observed Being free from the trying ordeal of egg development and oviposition the insect lives much longer, and, as our figures show, more than half the number of mosquitoes with which our batches are begun survive many weeks It is by using mosquitoes collected at this

period of the year that we have been able to prove that malarial zygotes and sporozoites are still active after the mosquito which harbours them has lived several months

One other point in the figures remains to be explained namely the drop in the percentage of survivals among mosquitoes caught in November and subsequent winter months These mosquitoes are those which have already lived as adults in nature in a hibernating or semi hibernating condition for some weeks or months according to the date when they are caught During this wintering life ovarian development goes on, but so slowly as to be inappreciable to the naked eye In ordinary circumstances in nature it goes on so slowly that the ova of wintering mosquitoes do not show signs of growth until April But the ova of some of these mosquitoes are evidently ready for this growth at any time from late November onwards for some of them begin to grow rather quickly when the mosquitoes are subjected to the artificial warmth of our incubator (23°C) Their development is not nearly so rapid as in mosquitoes caught in May and June but it is sufficient to cause some of the mosquitoes to undergo the peril of oviposition during the period of infection This causes the fall in survival rates during the winter months which the chart shows

Now in this description I have spoken only of the events which happen to mosquitoes in the artificial conditions of our laboratory After making the observations which I have described I searched the literature for any comparable observations in natural conditions I found them in the observations made by Prof Swellengrebel round Amsterdam where the habits of *maculipennis* are the same as they are in England During the years 1921 to 1923 Prof Swellengrebel made a monthly collection of female *maculipennis* from stables and examined the condition of their eggs The following statement gives the percentage of *maculipennis* in which developing eggs were found during each month of the year —

Percentage of female maculipennis *caught in nature with developed eggs*

January	0
February	0
March	3 9
April	20 0
May	27 0
June	24 0
July	31 0
August	17 0
September	1 4
October	0
November	0
December	0

Now, we can compare these figures with our figures showing the monthly per centage of mosquitoes which lived long enough to become transmitters of malaria This is done on the following diagram (Fig 2)

DURATION OF LIFE OF *MACULIPENNIS* AT DIFFERENT SEASONS

IMPORTANCE OF EGG DEVELOPMENT AND OVIPOSITION

Laboratory Results Compared with Findings in Nature

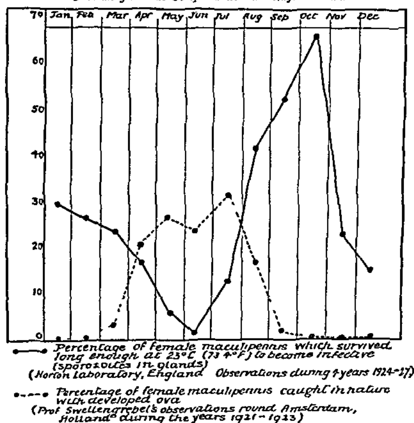


Fig 2

I think you will agree that Prof Swellengrebel's findings in nature confirm our view that the short life of *maculipennis* during the months April to July is due to egg development and oviposition

The lessons of these observations from the point of view of the spread of malaria seem to be (1) That in future we must endeavour to correlate the seasonal incidence of primary malaria, not with the seasonal prevalence of the mosquito concerned but with the seasonal prevalence of the individuals which live long enough to be transmitters In June there may be an enormous number of adult *maculipennis* in a malarious place, but if we know that during that month less than 2 per cent of them live long enough to become transmitters of the disease their abundance is not so important Obviously, it is much less important than a smaller abundance in August or September the simple calculation from our figures that 100 mosquitoes

in September are equal in importance to 3,000 in June does not by any means express the true difference because the September mosquitoes will live several months while the June mosquitoes will live at the most only a few weeks

The results of Prof Swellengrebel's dissection of mosquitoes caught in houses round Amsterdam during 1920 to 1922 enable the comparison to be made which is shown in the diagram (Fig 3) (2) If the process of egg maturation and

DURATION OF LIFE OF *MACULIPENNIS* AT DIFFERENT SEASONS

Seasonal Infection in Nature Compared with Laboratory Findings on Seasonal Duration of Life

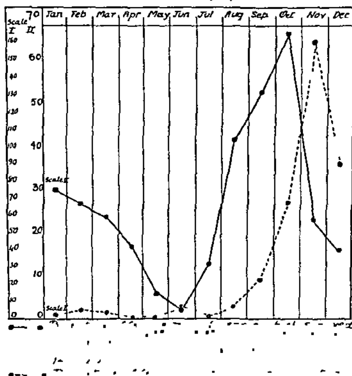


Fig 3

oviposition is such an important cause of death that it almost entirely prevents the transmission of malaria by Anopheles during the months of its occurrence, the number of broods that each species has in different localities and the period of the year during which maturation of eggs and oviposition occurs ought to be worked out much more carefully than has hitherto been attempted in many places The results may provide a clue to the explanation of some observations on malarial incidence which are at present obscure

PROGRESS TOWARDS THE REALIZATION OF BIOLOGICAL CONTROL OF MOSQUITO BREEDING

BY

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At present mosquito control is affected almost entirely by mechanical means either by drainage works *involving always considerable and often extremely heavy* capital expenditure with subsequent low upkeep charges or else by the application of larvicides which not only involve continuous costs for periodical application but often in addition a certain amount of drainage works to concentrate water within treatable limits

Beyond these methods there is at present only one method of true biological control which has been practised from earliest times —the use of larvivorous fish but *save in circumscribed areas such as wells* and cisterns where there is space in the ecology of nature for them to exist in sufficient numbers to be effective this method has neither yielded, nor promises to yield any useful results The hopes which have *sometimes been based on the method have proved extravagant for* in large bodies of water the natural enemies of the fish keep their numbers as elsewhere in nature within normal limits and control in the sense that connotes [illegible]

been extended to the bionomics of larvivorous fish at least in Asia with a view to increasing their efficiency

The same remark applies to various aquatic Rhynochota and Coleoptera many of which are extremely vicious predators on mosquito larvæ

The discovery by MacGregor (1921) of a connection between the hydrogen ion concentration of water and the species of mosquitoes breeding therein at once moved the problem on to another plane Here appeared to be a Pisgah sight of biological control of mosquito borne diseases not, as in the case just considered by attempting to influence the bionomics of animals as equally enwrapped in an ecological mesh as the mosquitoes themselves but simply by rendering dangerous waters unsuitable to breeding by means which though never specifically defined are none the less clearly visualizable It is of course a

truism known to every worker that in any area however malarious the proportion of the total extent of water used for breeding by carrier species is very small indeed Prior to MacGregor s discovery this fact had been accepted without search for an explanation though Watson (1921) had drawn attention to the phenomenon and had suggested that the explanation lay in something in the quality of the water ' which could be made use of for biological control Following on MacGregor s discovery however the search was taken up in several directions at once

Buxton (1924) made a series of pH observations in Palestine but their number was small and the results inconclusive MacGregor (1924) continued his English observations in Mauritius and reached the generalization that the Anophelines are alkaliphiles the Culicines generally acidophiles He also made the observation which followed on Watson s classic felted alga observation that dhobying and the discharge of sugar factory effluent into a stream rendered it sterile for a considerable distance but he does not seem to have tried to correlate this observation with pH determinations

The present author (Senior White 1926) published the first long series of records of larva pH findings made in the island of Ceylon and though for each species there appeared to be an optimum value the range found for the majority was very wide almost in fact that of the whole series of waters examined The conclusion is that only extremes of acidity and alkalinity are inhibitory but from an investigation of the residual pH that is the value after expelling CO by shaking or boiling it was found that for Anophelines at least acidity other than that due to CO is definitely inhibitory The first conclusion that only extremes of natural pH have any inhibitory effect has been confirmed by experimental work by Buxton (1927)

Failing thus to find the necessary explanation in hydrogen ion concentration the author in the same paper gave the results of the investigation of the values of dissolved oxygen total dissolved solids and saline ammonia in various waters Again it appeared that there were specific optima in respect of dissolved solids and that where sea water influence is concerned there are actual biological races of the various species in this respect which will be further confirmed when the results obtained by me when surveying the new Imperial Harbour at Vizagapatam are published In respect of dissolved oxygen it was found that in general a low content was unfavourable to most species and in rice fields it was shown that there was an apparently close relationship between rises in the oxygen content and the entrance into the fields of the carrier species *A funestus* Finally the tentative conclusion (for the number of observations was small and the method crude) was reached that saline ammonia in higher quantity than one part per million was absolutely inhibitory to the presence of Anophelines other than the *rossi* group

It should be mentioned that a year earlier than the commencement of the author s investigations Lamborn (1922) had published a few chemical analyses of

waters, but his results hardly suggest any factor as dominant Simultaneously with the present author's work in Ceylon, Hacker (1921) showed *A maculatus* and *A kochi* following, inversely and directly respectively, the albuminoid ammonia curve

Williamson (1926) continuing Hacker's work in Malaya, like Senior White and his predecessors, encountered little that was helpful in pH He also found extreme natural acidity inhibitory This author has made considerable study of the effects of peat, an opportunity vouchsafed to him alone, as peat is absent from all but very high elevations in India and Ceylon and his detailed results will be awaited with the greatest interest

The present author, again travelling over wider and more diverse areas of this country than any other worker on pH has been enabled to do elsewhere, made in 1925 26 a further considerable series of pH observations, not yet published which will again only show that there is nothing in pH *per se*, and that the optimum *values found in his Ceylon work are almost certainly only applicable to the country* at that time investigated, and would not apply elsewhere, thus answering in the negative the question propounded on this point in his paper of 1926

This year *pari passu* with the malaria survey of Delhi on which I am now engaged investigations have been undertaken into the following factors —Hydrogen ion concentration, 'residual pH,' dissolved solids, dissolved oxygen carbonates and CO_2, phosphates, saline and albuminoid ammonia

The area investigated is practically totally distinct from all other portions of the East where similar work has been done, though most of the species are of wide occurrence thereover The country around Delhi is typical of the vast expanse of the Indo Gangetic Plain

These investigations are as yet uncompleted and unpublished, but as they have been continued at the time of preparing this paper for seven months a brief summary of the results will perhaps be of interest —

(1) Hydrogen ion concentration of itself explains nothing

(2) 'Residual' pH is always alkaline, as found in Ceylon but as no natural pH of lower value than 7 0 has been found, this can hardly be said to confirm the Ceylon result

(3) Total solids in solution are generally very much higher than was found in Ceylon Optimum values are thus shown to be purely local, and are probably merely correlated to another, and controlling, factor

(4) Carbonates and CO_2 do not of themselves explain anything but, as I hope to show in a subsequent paper based on results obtained partly in Delhi (for alkaline soils) and partly in Ceylon (for acid soils) there are very high correlations between the 'movable carbonate' and the pH, and between the total carbonate and the conductivity Though this is what one would expect theoretically, it may serve as an indirect means of approximating the *carbonate* values the direct measurement of which involves titrations not very suitable for field work

(5) Phosphates investigated for their probable effect on the microplankton that forms the larval food have almost always been present in quantities sufficient to rule them out as a factor directly affecting the presence or absence of larvæ With their enormous and universal pollution bodies of water around a great city are not suitable areas for the investigation of this point I am however of the opinion that it may be of great importance in the economy of the stream breeders that cause hill foot malaria and are absent from the great plains

(6) Dissolved oxygen which yielded promising results in Ceylon streams has not done so here The amounts found by the Winkler process used have often appeared impossible for the very polluted waters examined The point was submitted to Dr W R G Atkins F R S perhaps the greatest authority on water biochemistry who after further consultation with Dr Ramsden of Trinity College Dublin, is of the opinion that the values are false and are due to nitrous acid released from nitrite when the HCl is added to the precipitate which itself also releases iodine from the iodide and thus stultifies the final titration As Dr Atkins has pointed out to me the Winkler process has seldom been made use of in the 'foul morasses which interest the malariologist

(7) Complete confirmation of my Ceylon result of the inhibitory effect of saline ammonia in higher concentration than 1 p p m has been obtained A series of 151 determinations made up to the date of preparing this paper shows only six exceptions Of these four belong to the *rossi* group the remaining two to *culicifacies* represented however by but three individual larvæ

The ammonia ratio discovery is not as I for long imagined new Waddell (1903) discovered that very small amounts of ammonia are fatal to mosquito larva I have not been able as yet to consult the original paper but I have failed to reproduce the fatal effect in the laboratory with eggs of the *rossi* group using concentrations far higher than anything ever found in Nature Advices of his latest unpublished work by Williamson indicate that the true inhibitory effect is not ammonia *per se* but the ammonia nitrate ratio This may explain the failure of experiments to confirm observed facts The investigation of the point for India is about to be commenced but too late to yield results this season If confirmed I think that the apparent relationship with dissolved oxygen found in Ceylon falls into line with the discovery

The bearing of these discoveries by Waddell whose claim to priority I am thus very glad to bring before this meeting the author and Williamson on the nitrogen cycle in water with reference to Anopheline breeding promises at last to lead to a practicable method of control applicable at least to standing water breeding grounds though not to swift streams or hill foot seepages In the nitrogen cycle proteids (determinable as albuminoid ammonia) are broken down to ammonia by a great variety of saprophytic bacteria but from that point the organisms concerned in the cycle are specific *Nitrosomonas* alone can convert ammonia to nitrites and *Nitrobacter* alone can carry the process forward to nitrates Now following on the original work of d'Herelle Gerretsen Gryns Sack and Sohngren

(1924) have isolated a bacteriophage for a nitrifying organism, *B radicicola*, and there is every hope by a modification of their method of similarly isolating bacteriophages for *Nitrosomonas*, and *Nitrobacter* if required, whereby the ammonia nitrate ratio should be regulatable at a concentration inhibitory to the breeding of carrier Anophelines The Dutch authors, moreover, have shown that their product is highly resistant to heat and desiccation, engendering the hope that a breeding place such as a depression that dries for part of the year, once inseminated, would remain more or less permanently sterile

REFERENCES

MacGregor (1921) .. .. The Influence of the Hydrogen ion Concentration in the Development of Mosquito Larvæ *Parasitology* XIII, p 348

Buxton (1924) . . Applied Entomology of Palestine, being a Report to the Palestine Government *Bull Ent Res*, XIV p 289

Idem (1927) . 'Researches in Polynesia and Melanesia', p 153

Watson (1921) .. 'The Prevention of Malaria in the F M S', (2nd Ed) Chap XIX

Lamborn (1922 Some Problems of the Breeding places of the Anophelines of Malaya, a Contribution towards their Solution *Bull Ent Res*, XIII, p 1

Hacker (1924) .. 'Report of the F M S Malaria Bureau', 1923, p 2

Williamson (1926) .. . *Ibid*, 1925, p 17

CHEMICAL FACTORS IN RELATION TO ANOPHELINE BREEDING

BY

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THE problems which chemistry, and especially the chemistry of the future, has in common with anti malarial science are of profound interest The past thirty years have brought many facts to light in connection with malaria and the mosquitoes which carry it, but they have also raised many questions which still await an answer One of the most important of these is why the larvæ of different species of Anophelines have different habitats Before we can answer this question we must have a precise knowledge, physical, biological and chemical, of mosquito breeding waters embracing all relevant details and to attain them, masses of irrelevant data will have to be collected sorted out experimentally tested and rejected Only with increased knowledge of the causes which determine the suitability of particular types of water for particular Anopheline species may we hope to attain complete mastery of the situation Another equally interesting problem the solution of which may enable us to regulate the abundance of malarial parasites, and thereby the risk of infection is why certain species of mosquitoes differ from others in their ability to accommodate the parasite cycle and to transmit malaria Indeed we may profitably extend enquiry, and ask why among all the blood sucking arthropoda mosquitoes only, and apparently among them only a comparatively few species of Anophelines, are efficient vectors If it can be shown, and I think there is evidence for the fact, that there is often a correlation between the type of water in which dangerous Anophelines breed, and the fact that they are efficient carriers of malaria, there will be additional reason for the study and precise definitions of breeding waters

I believe that biological chemistry will throw a great deal of light upon both of these problems namely, that of the adaptation of larvæ to the water in which they are found, and that of the malarial parasites to their hosts both insect and human But up to the present its aid has not been sought, and in the present paper I can only very incompletely present a few arguments based upon the few facts gathered by volunteer workers, which may substantiate its claim for recognition as an integral and essential department of anti malarial research, so much so that any country which undertakes to investigate malarial problems without providing for a biological chemist of proved ability and originality is minimizing the good that should result from investigations in other fields of malariology, and betraying its trust

To consider first the records of breeding places a great deal of extremely suggestive and as far as it goes valuable information has been gathered in past years Major Covell has earned the gratitude of all anti malarial workers by summarizing this information and relating it to the known facts concerning the ability of the various Anopheline species to transmit malaria Field observations are a first aid to the diagnosis of breeding locations But they necessarily lack precision For the most part they relate to topographical details and the information volunteered regarding the constitution of the water itself is confined to such statements as that it is brown peaty clear or muddy, pure or fouled running or stagnant and finally whether it is deep or shallow, while often recorded details of value are whether the surface is exposed to the sun or shaded and whether the water presents an extended surface or is circumscribed in small or large pools As will be shown many and doubtless all of these facts are in direct relation to chemical factors some of which are apparently of great importance in determining the presence or absence of Anopheline species Records of aquatic vegetation usually stop short at stating whether or not it is present or is scanty or abundant

Grass or reeds are sometimes mentioned and field observers can do no more Unless they are botanists familiar by long residence with the country they are working in the identification of particular grasses or reeds or submerged plants cannot be expected of them But even general records are instructive The fact of free illumination together with that of the presence of abundant vegetation justifies the inference that photo synthetic activity is producing both an abundance of organic food and of oxygen which is of value in purifying the water And exposure to the tropical sun sometimes raised the temperature of the water in Malay to 98°F or nearly 37°C Preliminary experiments made in conjunction with Mr Gater showed that larvæ of most of the Anophelines tested were killed by three hours' exposure to slightly higher temperatures few surviving at 40°C In one experiment all larvæ of *A sinensis* and *A barbirostris* were killed at temperatures of 39 C and above and all larvæ of *A aconitus* at 37 5°C and higher temperatures while in another experiment three larvæ of *A leucosphyrus* were all killed at 36 C Therefore the high temperature of open water may possibly be a factor excluding from it species which breed in the shade or in running water For this and many other reasons it is necessary to guard against assuming that chemical factors are the only ones which operate in determining the distribution of Anophelines To do so would be to take a very narrow view of the facts and one contrary to common sense For apart from physical factors which affect the larvæ a host of environmental circumstances influence or prejudice the well being of adult mosquitoes Of these shelter atmospheric conditions, especially humidity and access to blood are among the most important

The most conclusive evidence that certain waters may be definitely destructive to Anopheline larvæ has been obtained by Purdy He introduced female

Anophelines into a large cage placed in an Anopheline sterile rice field and it was found that the resulting larvæ survived only two or three days Similar but less convincing results were obtained in the area in Krian from which Anopheline breeding is absent by placing larvæ of various species in floating muslin cages In both these cases stagnation and rot were present Purdy records the presence both of Euglena and of a blue green alga In the particular Krian fields here referred to one of the Euglenidæ namely a Trachelomonas formed scums constituting a brick red to greenish water bloom The species most commonly found all over Malay resembles *T wermeli* var *paludosa* Skvortzow, and it is occasionally associated with larvæ of *A sinensis* when these are present in fouled water the larvæ probably feeding on it The interpretation of these facts is instructive as indicating the relation of Anopheline distribution to biological factors I think none of specialized and obligatory importance has ever been proved to exist except disease producing micro organisms such as *Lambornella stegomyiæ* described by Keilin and *Saprolegnia sp* recorded by MacGregor as destructive to larvæ of *A maculipennis* and *A bifurcatus* etc It is however probable that bacteria both those directly productive of disease and those able to cause food poisoning exist in stale and putrescent water and effectively inhibit the breeding of pure water species in extreme cases putting a stop to all mosquito breeding They may possibly also generate soluble poisons Harvey has shown that these arise in sea water from the bacterial decomposition of peptone and are effective after passage through a porcelain filter But there are good grounds for believing that mosquito larvæ are not very susceptible to dissolved poisons Teichmann s figures for larvæ of *C fatigans* prove that the larvæ succumb as quickly to the presence of this gas as to that of dissolved cyanides when the concentration of the gas is about a hundred times less than that of the latter taking the average of his own and other experimenters data for cyanides In other words the rate of absorption of even a highly diffusible poison is about a hundred times less by way of the skin and mouth combined than by way of the breath ing orifices The amount of liquid taken in by the mouth is very small when larvæ are well fed their guts being packed with solid food as though by a ramrod Nevertheless they may be killed both by soluble poisons such as cyanides and arsenious acid and by poisonous solids But they are much less vulnerable through their chitinous cuticle than by way of their orifices And it is not without significance that the two commonest agents of destruction namely oil and copper arsenite gain entry respectively through the tracheal openings and the mouth The trend of the argument is therefore that natural poisons present in water will be effective in proportion as they are absorbed by the mouth and that poisoned solids such as contaminated food particles, have the greater chance of effectiveness

With regard to visible associates of Anopheline larvæ such as algæ, or forms which usually occur dissociated from larvæ of particular species, a specialized correlation positive or negative, is not to be assumed without proof Aquatic

vegetation has an important influence upon the water, but this influence is generally not specific The case recorded by Senior White of the invariable association of *C bifurcatorhynchus* with certain *spirogyras* is certainly exceptional, and may perhaps be presumed to be due to a food preference of the former But plants characteristic of certain soils and types of water and indicating somewhat vaguely the probable presence or absence of particular species of Anophelines may readily be distinguished The *Euglenidæ*, including *Trachelomonas*, well illustrate this point They require for their culture peptone, fish extract, asparagin, ammonia and other substances resembling those present in fouled stagnant water Their presence absolutely contra indicates pure water species of Anophelines such as *A maculatus*, or even *A aconitus* But the range of adaptability of *Trachelomonas* overlaps that of *A sinensis*, for it is often found, and attains its maximum development in water which is too stagnant and foul for this species, or even for culicine larvæ These facts are illustrated by the analyses of the Anopheline sterile Krian waters referred to above, which are tabulated where their constitution may be most conveniently considered in relation to other types of water Certain of the blue green algæ appear to have a similar but even greater addiction than that of *Trachelomonas* for fouled water This is the case for example with *Microcystis* which forms an oily green film consisting of mucilaginous packets of blue green cocci on very dirty water I have fed it to mixed Anopheline larvæ without apparent ill effect, so that the reason for their absence from water which contains it must probably be sought for among chemical and chemically induced bacterial inhibitive factors The mechanical properties of the scum, however, probably inconvenience larvæ, and the size of the larger packets which they cannot swallow, and have to toss off, appears to irritate them

The only record of mineral factors (except salinity) of significance with which I am acquainted is Senior White's inference as to the unsuitability of water rendered alkaline by magnesium The scarcity of larvæ in certain volcanic districts in Java, where abundant growths of *A zygnemæ* should render the water suitable to them, may possibly be attributed to similar causes, though the effect of cold night temperatures, at the elevation of Garoet, for instance, must not be overlooked Dr Scharff, who, I hope, may be present in order to confirm the statement has found that *A maculatus* is commonly substituted by *A karwari* on laterite soils, while the former occur on granite formations The latter fact appears to be generally true of Malay, but not exclusively so, if *A maculatus* also occurs on limestone formations Whether the presence of potash derived from the felspathic clays which result from weathering of granite contributes sustenance to some problematic food organisms, possibly diatoms or desmid, must remain a moot point The generality of mineral solutes being present in concentrations of millionths or fractions of a million can only be of indirect importance in so far as they provide the food of aquatic vegetations Some tests of the phosphate content of a variety of waters in the F M S may be quoted Phosphates are essential food for algæ, and Atkins has shown that depletion

of phosphates in summer is a cause which limits their growth both in salt and fresh water The two highest findings were 2 parts per million of PO_4 associated one with a heavy growth of moss in a split bamboo, where *A watsoni* and *A leucosphyrus* Hackeri were breeding and the other with a dense growth of spirogyra found in a ditch for which larval records are lacking On the other hand, a massive growth of a filamentous blue green alga resembling *Lyngbia*, was present in a ditch where no phosphate could be detected, but there had been very heavy rain It is of interest that *A kochi*, a species whose larvæ are commonly found in ditches and puddles with scant or invisible algal growth, was present in water where there was a barely detectable trace of phosphate, amounting to less than one in ten millions of PO_4, and macroscopic algæ were absent

Nor could phosphate be detected in a highly ferruginous patch of water in a fallow rice field which yielded only one Anopheline larva in association with hundreds of culicines Phosphate varying from one in three millions to one in ten millions of PO_4 was found in all but one of the remaining Anopheline-containing water, the species being *A fuliginosus*, *A sinensis*, *A barbirostris*, *A rossi* Giles and *A vagus* in association with the three last named, and *A leucosphyrus* (one in ten millions) found in a slightly peaty jungle pool The exception was in a spring where *A maculatus* was present Excluding *A leucosphyrus* and *A vagus*, the other species named breed in marshes or large pools and their association with detectable phosphate is in harmony with their general preference for water rich in vegetation

The absence above recorded of phosphates from the ferruginous water is perhaps to be expected owing to precipitation of ferric phosphate, but this cannot be assumed with certainty, since iron is present in stable and probably organic combination in most natural waters This is the view advanced by Ellis in his book on iron bacteria, and it is supported by the fact that the salts present are not freely ionized in most cases giving ferro and ferri cyanide reactions only slowly upon addition of acid It has been very generally assumed that iron is the factor in ferruginous waters, which unfits them for Anopheline larvæ. Culicine species may, however, be found, as in the case cited, in deep brown water, with or without bacterial films, and even with ferrous iron present The opinion that iron is directly toxic to larvæ is perhaps an example of the fallacy of confusing a discriminant of conditions unfavourable to breeding with the con ditions themselves For filmed and highly ferruginous water is always stagnant, contaminated by root rot and other vegetable rot, and de oxygenated, as is proved by its liability to contain, besides appreciable quantities of ferrous iron very large quantities of ammonia, which is rapidly converted into nitrate on removal from the soil On one occasion I obtained a dense precipitate with Nessler's solution, indicating a concentration of ammonia probably equivalent to con siderably more than 10 in a million of nitrogen Even in this water, though even culicines were absent, blood worms (*Chironomus sp*) were taken That

iron is directly or indirectly unfavourable to most larvæ is probably true For not only does it militate against algal growth but, as Nelson and Kerr(1) who are criticized by Zajdel and Funk(2), have claimed, colloidal solutions of ferric hydroxide absorb vitamins *which may thus be precipitated* Experiments conducted for me by Mr Basmin, however, showed that concentrations in their own water of ferric alum killed practically all larvæ of *A sinensis*, *A barbirostris* and *A fuliginosus* within 5½ days at a concentration of 100 parts per million of iron, and 50 per cent of the *A sinensis* and *A barbirostris* at a concentration of 10 parts per million, with good survival among the controls, while 70 per cent of larvæ of *A separatus* survived 100 parts per *million in an experiment lasting 4¾ days, and in another, 60 per cent lived 5½* days On several visits to Port Dickson, I made a study of the iron content of the very remarkable water in which these larvæ of *A separatus* were breeding When diluted by rain, the analyst found 25 parts per million of iron, and in the preceding period, when the water was much more concentrated, heavy precipitates of both ferrous and ferric iron were obtainable by ferri and ferro cyanides, even without addition of acid By somewhat rough colorimetric *methods I found a concentration approaching if it did not exceed, 100 parts per* million of ferrous and ferric iron added together The pH of the water was about 3 0 It tasted acid and immediately furred the palate and set the teeth on edge It is therefore evident that these particular larvæ, which were always to be found and were quite abundant, tolerated both a very high degree of acidity and a high concentration alike of ferrous and of ferric iron The ditch in which they occurred was situated in a rubber estate on flat coastal land and was carpeted with dead leaves *A scanty growth of unidentified green algæ was attached to the side* of the ditch but dissection proved that they formed a little or no part of the larval food, the guts being full together with a few included cells, of amorphous brown material containing both ferrous and ferric iron This type of gut content is very common all over Malay, but I cannot make any precise statement as to its content of iron I would take this opportunity of expressing my indebtedness to Mr R Blair for having made an analysis of the above mentioned water, as well as the analyses quoted in the attached table, some of the averages compiled, however including the results of his labours for Doctors Lamborn and Hacker His analyses and also field tests show that iron (predominantly in the ferric form) is usually present in Anopheline waters in concentrations of less than one in a million, but vigorous breeding was associated with concentrations *up to 6* parts per million (*A aconitus*)

Before considering the table of organic and organically derived factors, the question of hydrogen ion concentration in relation to Anopheline breeding calls for comment, and pH data are included in the table If pH values were an important determinant, the fact might invalidate conclusions based upon the tables But the facts are that all the species extensively studied which are not specialized in one particular type of water occur indifferently over wide ranges of pH

or are found at the extremes These facts do not consort with the view that any concentration of hydrogen ions ordinarily met with *per se* inhibits or is even appreciably unfavourable to the breeding of at least the commoner Malayan Anophelines And when through limited observation they appear to do so we still have to take into account all the other factors which determine or are associated with pH values Many of these are factors to which mosquito larvæ are indifferent so that it comes about that when the different types of water in which the same species is found breeding are surveyed even discriminative value is not to be expected of pH readings And I think the following observations would contradict the expectation if it existed *A umbrosus* breeds at a pH of about 4.5 but I have found it fairly abundant at 6.7 *A barbirostris* exceptionally abundant at 5.3 and abundant at about 8.0 *A separatus* exceptionally abundant at under 3.6 The lower values are the lowest yet recorded for the species Since sudden discontinuities are contrary to the rule of Nature it cannot be supposed that the observed limits are very near the extremes of tolerance especially when they are associated with exceptionally prolific breeding And when for example *A barbirostris* is absent from rice fields with a lower pH than 6.0 the fact must be attributed to associated factors such as poisonous food or solutes lack of proper food or the presence of harmful micro organisms which under the particular conditions existing a low pH may favour Also as has been seen there is reason to suppose that mosquito larvæ are not particularly susceptible to solutes under which category hydrogen ions may be classed when considering their possible direct action

Evidence that gases normally present in water are directly either harmful or beneficial to mosquito larvæ is lacking Sulphuretted hydrogen can rarely if ever, be detected what is formed in the soil being oxidized to sulphuric acid by sulphur bacteria And as Harrison and Subramaniya Ayer have shown associated algæ and bacteria forming soil crusts can oxidize hydro carbons Of these even acetylene is non poisonous I found that larvæ of *A maculipennis* though rendered inert, recovered completely from the effect of high concentrations More or less carbon dioxide is usually present in solution and as Senior White has pointed out lowers the pH of the water without harmful effect In exceptional cases I have observed a rise of 0.6 and in one case of over 1.0 Sorensen degree on aeration with numerous larvæ respectively of *A barbirostris* or *A sinensis* present Since the skin the gut and as Soni and others have shown the stigmatic openings are alternative routes for excretion of carbon dioxide the presence of even a super saturated solution need not seriously incommode larvæ Unaccustomed salinity has been shown to cause a reversal of the ordinary route and passage out of the gas through the siphon in *C fatigans* instead of through the skin With regard to the oxygen although its absence is an unfavourable sign because it indicates pollution of the water, what is in the water is not needed for respiration and when larvæ are found in super saturated water among floating algæ although they may doubtless benefit by their situation and by the purifying effect of the oxygen

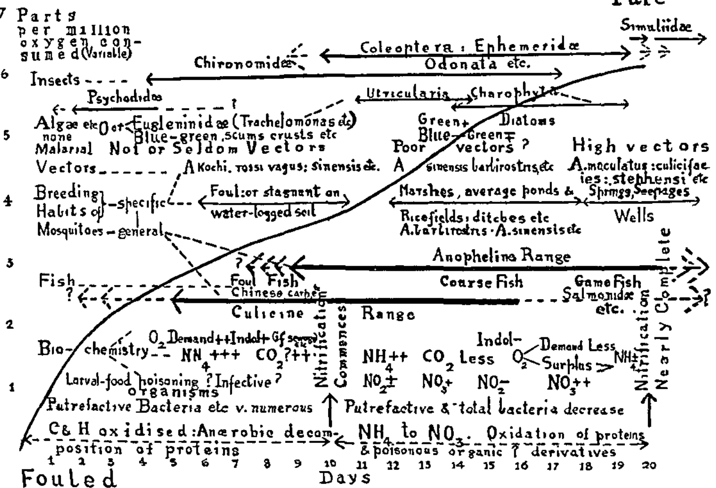

Pure
Fouled
7 Parts per million oxygen consumed (Variable)
6
5
4
3
2
1
Simuliidæ
Coleoptera: Ephemeridæ
Chironomidæ
Odonata etc.
Insects
Utricularis
Charophyta
Psychodidæ
Algæ etc
0 or
Euglenidæ (Trachelomonas etc)
Blue-green, scums crusts etc
none
Green + Blue-Green ∓
Diatoms
Malarial Vectors
Not or Seldom Vectors
Poor vectors ?
High vectors
A Kochi, rossi vagus; sinensis etc.
A sinensis barbirostris etc
A. maculatus: culicifacies: stephensi etc
Breeding Habits of Mosquitoes
specific
general
Foul: or stagnant on water-logged soil
Marshes, average ponds & Ricefields: ditches etc
A. barbirostris · A. sinensis etc
Springs, Seepages
Wells
Anopheline Range
Fish
Foul Fish
Chinese carbe
Course Fish
Game Fish
Salmonidæ etc.
Culicine Range
Nitrification Commences
Nitrification Nearly Complete
Bio-chemistry
O_2 Demand ++ Indol + Gf (?) etc
NH_4 +++ CO_2 ?++
NH_4++ CO_2 Less
NO_2± NO_3+ NO_2− NO_3++
Indol−
O_2 Demand Less
Surplus > NH_4±
Larval-food poisoning ? Infective ? organisms
Putrefactive Bacteria etc v. numerous
Putrefactive & total bacteria decrease
C & H oxidised: Anærobic decomposition of proteins
NH_4 to NO_3. Oxidation of proteins & poisonous organic ? derivatives
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Days

Fig 1

upon the water, there is no proof that it exerts a direct physiological action upon them

A discussion of the influence of organic factors upon Anopheline larvæ would lead into speculation the merits of which future research alone can decide But the accompanying table goes to show that correlations exist between certain of these factors and Anopheline breeding each species having its more or less extended range of tolerance Dr Hacker first drew attention to the preference of *A maculatus* in contrast to *A kochi* for water having a low albuminoid content And in extension of his observation the table shows that the same factor is important for other species as well *But it does not stand alone A large absorption of oxygen* from acid permanganate indicative of high general organic content is similarly *correlated, and the degree to which nitrification proceeds shows an inverse* correlation with the above factors and a direct one with the breeding of pure *water species The interpretation suggested is that the essential fact is the condi*tions under which proteins undergo decomposition Under conditions favouring *the formation of nitrates and accompanied by a low content of both albuminoids* and other organic matter the water is favourable to pure water breeders The *marsh and rice fields'* group exhibited in Column II are intermediate in their preference between typically pure water species such as *A maculatus* and these like *A kochi* which are tolerant of stagnant shallow water undergoing little oxidation and in which a small volume is contaminated by a relatively large amount of vegetable débris In the next category IV is definitely foul water the test of foulness not however being the amount of albuminoid matter but the presence of deleterious substances and possibly harmful bacteria associated with them and which with efficient oxidation would be eliminated The ratio of the amount of nitrate present to either the content of ammonia or of protein appears to be a rough but not inefficient indicator of the degree of purity of the water as thus defined

Coming to practical applications it is very generally recognized that only a few species of Anophelines will tolerate sewage contaminated water That they can also be differentiated by their degree of tolerance of vegetable rot was first suggested by Sir Malcolm Watson in connection with his observations in the Krian rice fields when he proposed that dangerous species of mosquitoes might be abolished by the rotting of some fibre This is what happens in Krian which has a peaty basis and where even in the purest water to be found only *A sinensis* and *A barbirostris* breed with a few *A kochi* in pools The rotting of heavy crops of reeds prevents breeding before the rice crop and even during the cropping season a large area covering many square miles so far as observation goes is free from mosquito larvæ (*vide* II 5)

The question therefore arises whether these conditions can be imitated without achieving the complete absence of mosquitoes the columns from II, I to 5 seem to indicate that there is the possibility of changing the Anopheline fauna by regulating the amount and kind of organic matter in the water As Sir Malcolm Watson long ago suggested it should then be possible to say to this species of

TABLE

Showing water classified by predominant larvæ and graded according to purity and presumed degree of oxidation of organic nitrogen

(*Averages in parts per million—Analyses by Mr R Blair*)

GRADE	I	II					III	IV
Group description	1 Spring	1 Marsh ditch and rice field	2 Pond, marsh, ditch	3 Rice field pond, ditch	4 Pond, rice field	5 Peaty rice field	1 Ditch in rubber	1 Pond fouled by pig stye
Number of analyses	3	8 (6 recorded as slowing)	5	6	5	2	7	1
Predominant larvæ	*A maculatus* alone	*A aconitus*	*A barbirostris*	*A fuliginosus*	*A sinensis*,	None	*A lochi* 96 per cent *A maculatus* 4 per cent	*A sinensis* alone with Trachelomonas
Oxygen absorbed in 3 hours from acid permanganate	(1) 0 395	2 833	*Organic* 5 2404	0 783	8 170	7 572	Dr Hacker's *collection* 88 269	3 572
Albuminoid nitrogen by alkaline permanganate	0 057	0 208	0·470	0 531	0 612	0 460	2 66	0 100
Ammonia N	0 057	0 02	*Organically derived* 0 057	0 060	0 048	0 062	0 300	0 024

Oxidized (nitrate) N	(2) 0·395	0·035	0·148	0·026	0·048	0·030	0·137	0·009
Ratio of $\frac{\text{nitrate}}{\text{ammonia}}$ N (average of individual ratios)	8·8	2·06	2·07	0·84	0·80	0·61	0·47	0
pH average of samples analysed. Record lacking for some	5·3	6·5	*Of varied origin?* No record	6·2	5 to 7·5	About 5·0		·
pH range	5·0 to about 8·0 probably 4·5 to 8·0	5·0 to ? 8·0	5·3 to 8·0	6 to 7	5 to 8 probable		·	··

EXPLANATION OF TABLE

1. This is a rough index of the total organic content.

2. *Nitrites*—A trace in one *A. fuliginosus* sample only.
Nitrates—To be discounted in the exceptional case when performed in the soil: they are absorbed by green vegetation and are re-formed from ammonia derived from protein lysis only if oxidative, etc., conditions favour nitrification.

3. Discrepancy due to averaged ratios 7·15, 3·33 and 2·86 contributing disproportionately little to the total of ammonia and nitrate. These ratios from marshes and a flowing ditch are probably more nearly typical than lower ones from rice fields where larvæ may have been washed in from irrigation ditches; but one marsh sample from running water containing *A. aconitus* contained no nitrate.

4. IV appears to indicate that absence of nitrification is a better index of recent fouling than high content of either albuminoids or of ammonia.

5. I and III show the extremely wide range of adaptability of *A. maculatus*. Is this species an equally good carrier of malaria when bred from either extreme type of water?

6. II, 5 fails fully to reveal the reason Anopheline-breeding is absent from these fields. It may tentatively be attributed to defective oxidation operating in conjunction with acidity; but the operative, possibly bacterial, causes are undetermined.

7. II, 3 and 4 do not discriminate between *A. sinensis* and *A. fuliginosus*. The latter tolerates recent vegetable decomposition but, unlike *A. sinensis*, it is extremely intolerant of peaty soils and of animal foulings. Its natural pH range may not improbably extend to a higher alkaline limit than yet recorded.

8. Dr. Hacker found that larvæ of *A. aconitus* were much more frequently associated with those of *A. barbirostris* than with those of *A. sinensis* or of *A. fuliginosus*, a fact which the figures in column II, 1 to 4 seem to explain.

WHY DO ANOPHELES LARVÆ FEED AT THE SURFACE, AND HOW?

BY

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THE Anopheles larva is clearly adapted for feeding at the surface. This is shown by the palmate hairs which help to maintain it at the surface and its ability to rotate the head through 180°. If it were merely that the Anopheles larva maintained itself at the surface and fed there in the same general manner as a Culex we might think this was because a siphon had not developed or *something of the sort*, but that the larva turns its head to feed shows that it gets some advantage from feeding actually against the surface film. We studied what advantages there might be (*a*) in the way of extra food supply, (*b*) in the mechanics of currents produced, etc.

When freshly fallen rain water is taken from a pool and the surface examined, only some minute particles, many of them refractile, are seen on the surface. Many of these are particles of silica which appears rather easily to become captured in the surface film. One may see an occasional flagellate either attached to particles or swimming freely and occasionally a stray ciliate swimming near the surface. Within 24 hours a great change has taken place: bacteria arranged in curved rows in beautiful patterns have practically covered the surface. This bacterial film develops in certain waters very freely and at least three or four species of bacteria are seen taking part in it. That at least some of these organisms habitually grow in this way on the surface of waters is suggested by the regularity and extent of this growth in symmetrical lines and patterns over the surface. As growth proceeds the film thickens and may become visible to the naked eye. As the bacterial film develops flagellates, many of them resting and attached to the surface, become very numerous. Later, ciliates may appear in very large numbers.

This bacterial film is readily studied by dropping a perfectly clean cover glass on the surface of the water, removing and staining, etc. Twenty samples of water from small pools a few days after rain all showed a bacterial film more or less of the above type.

Larvæ we found can feed and apparently nourish themselves on such a film *But in doing so, they feed in rather a special way* *The current normally produced* by the working of the fans has little or no effect on the film and none of the film material is taken in until its continuity is broken by the larva, when patches of film may be dragged in by the stroking of the fans The larva may now turn its attention to dragging in the film in this way appearing to elevate its head slightly and keeping up a rather slow movement of the fans We have called this *film feeding* Though larvæ appeared to be able to nourish themselves in this way and grew and underwent ecdysis more or less in normal time they did not give one the impression that this was their normal method of feeding In fact when the film reached a certain thickness and consistency, it was obviously prejudicial to feeding The formation of a bacterial and flagellate film does not appear then to be the reason why Anopheles feed at the surface It may however on the contrary be a reason why some waters are unsuitable

Larvæ, when a bacterial film is not hindering them, begin and continue to feed in a very characteristic manner which we have called *free feeding* The fans are worked with a rapid rhythmical almost vibratile movement and extremely active currents are set up near the surface All particles lying just beneath the surface film as they come within the range of the currents are swept towards the mouth Such particles especially if any bacterial film is present can be seen passing beneath the actual surface film which is little if at all disturbed It appears to be this sub surface layer from which the larva normally derives its food

It was observed by focussing the microscope on the surface of different samples of water that there is a considerable tendency for particles of matter living and dead to accumulate just under the surface without making actual contact with the surface film Any inert substance lighter than water will obviously sooner or later take up this position It was also observed that flagellates ciliates and algal organisms had a tendency to collect in largest numbers in this position It would seem, therefore, that the object of the larva in feeding at the surface is to tap this special food supply

In '*free feeding*,' particles can be seen commencing to move towards the larva from a distance of at least the larval length (Plate XXVI fig 1) The depth of this current was estimated and found to be not more than about the thickness of the larval head (Plate XXVI fig 2) It is clearly therefore a very shallow and superficial disturbance of the water

At first only incoming currents could be made out but by employing suitable devices outgoing currents at right angles to the head on either side were detected These are normally freed from particles and so practically invisible They are powerful rapid circumscribed currents like the gulf stream leaving the Caribbean Sea They are caused by the main incoming current being deflected by the smooth outer surface of the mandibles which are kept closed

When feeding, the maxillæ and submentum make contact and even protrude from the surface film They thus block all backward exit for incoming currents i e ,

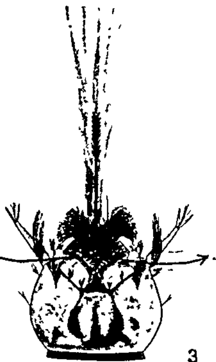
all passage except laterally under the maxillæ The maxillæ are kept in constant vibratile movement and comb the current as it passes beneath them The water thence striking the mandibles is shot out at right angles as described

Two whirlpools or eddies are formed on either side between the incoming and outgoing currents as will be clear from figures 1 and 3 (Plate XXVI)

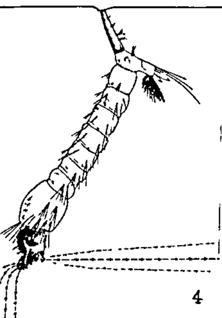
In Culex it is interesting to observe that a different type of water movement is set up Here water is drawn up from below and passing through the maxillæ is shot out parallel to the surface behind the larva (Plate XXVI fig 4)

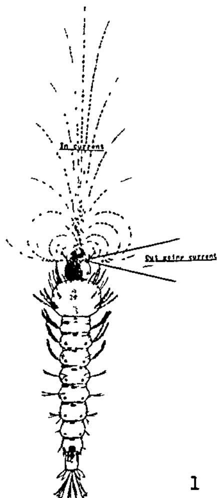
Substances in the sub surface layer which are objectionable to the larva may *considerably impede feeding Lycopodium spores were found to have this effect* Accumulating in the sub surface layer and being hard and difficult for the larva to swallow feeding was rendered almost impossible

The maxillary brushes can remove even such small particles as *Bacillus coli* When care was taken to deal with pure bacterial cultures (*B coli*) in the absence of amœbæ etc larvæ did not thrive Even with pure algal culture they did not thrive so well as with a mixed bacterial and algal emulsion









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EXPLANATION OF PLATE XXVI.

Fig 1 Dorsal view of an Anopheline larva. Dotted lines indicate the water current produced while feeding

" 2 Lateral view of an Anopheline larva feeding at the surface of water Dotted lines indicate the currents set up by the larva

" 3 *Head of an Anopheline larva showing the water currents produced while feeding* The arrow indicates the course of the in current and the two outgoing currents.

" 4 Lateral view of a Culicine larva showing the water currents (in dotted lines) produced while it is feeding.

INITIAL SEASONAL APPEARANCE OF MALARIA IN A SELECTED AREA IN INDIA, DEMONSTRATED BY PRESENCE OF PARASITES IN THE INSECT CARRIER

BY

BRUCE MAYNE

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THE particular part of the year in India known as the malarial season usually occupies four months and except in Burma, Assam and southern India, when it occurs earlier, the period is regarded as August to November.

In this connection some writers give two periods of incidence in the endemic malaria centres: a minimum incidence, a sharp short rise of malaria following the *early heavy rainfalls of March, April and May, and a maximum incidence toward* the end of and shortly after the longer rainy season. The first of these, the minimum incidence, is regarded as due to relapses from the previous season's residual infection. This period would tend to be subjected to more strictly local environments and is not as stable in its manifestations.

The rise in the course of malarial incidence reaches its peak towards the end of the rains and constitutes the usual long period of maximum density co-ordinated in biological sequence with mosquito propagation.

The observations of Bentley upon the influences of temperature and humidity on the malaria incidence of Bombay between the years of 1909 and 1911 brought to light a definite relationship between months of heaviest infections and the phenomenon of relative humidity. He found that relapses of malaria occur at the time of maximum heat and the occurrence of new infections coincides with a period *of slightly lower but almost uniform high temperature in the presence of* increased humidity. Bentley's investigations indicate a seasonal prevalence of infection among mosquitoes and the occurrence of fresh infections in man.

James in his report of anti-malarial operations at Mian Mir in 1902 narrates conditions relative to seasonal incidence, which are applicable to the present time:

In the month of March adult Anopheles were difficult or impossible to detect in houses. This persisted until the middle of May and from this time onwards to September and beginning of October adult insects increased steadily to a maximum. In November the numbers began again to diminish rapidly.

The seasonal prevalence of malaria corresponded very accurately in James's report with the prevalence of Anopheles The commencement of the season of new infections was noted a month after *Anopheles culicifacies* were found in houses on May 20th Thus allowing ten days for the parasites to develop to the sporozoite stage, and twenty days for the necessary incubation period in man, James gives the earliest time at which new infections could occur as the latter part of June During August to October the abundance of Anophelines and the endemic index of the bazaar increased together, so that in November with the almost sudden disappearence of adult Anopheles the endemic index quickly fell

The following report of a study pursued to determine the initial seasonal appearance of malaria and some factors influencing it, is confined to the district of Saharanpur in the United Provinces The Saharanpur district offers to the student of malaria an equitable cross section of the conditions contributing to the malariology of India, particularly the north central portion It has a North latitude of 30° and a longitude of 78° East The town of Saharanpur itself is completely surrounded by groves of fruit trees and areas cultivated in rice, wheat, and sugar cane The faunal conditions are probably influenced by excessive irrigation and defective drainage causing water logging of the soil during the rainy seasons although the annual rainfall rarely shows a maximum of forty inches

The investigation of the *initial appearance of infection in* the Anopheline mosquitoes of this representative region was conducted from the latter part of February to the latter part of September of the present year (1927) The work consisted essentially in the collecting and dissecting of the common Anopheles from four villages within a radius of three miles of the town of Saharanpur The incidence of malarial fevers was determined in a splenic index of village children and a superficial parasite index sufficient to indicate the infection risk of persons residing in the district These examinations were made at the beginning of the investigation and enough data were obtained merely to ascertain the infective material available The index of spleens was completed before the end of June from children of the 4 villages contributing the bulk of the mosquito collections A splenic index of 42 6 was obtained from an examination of 324 children The blood examinations from which the parasite index was obtained represents 155 individuals giving 62 5 per cent of positives There were 77 cases of malignant tertian with gametocytes amounting to 11 per cent, and 25 cases of benign tertian with 60 per cent gametocytes demonstrable

Dissections were made of the five predominant species of Anopheles namely, *culicifacies, subpictus, fuliginosus maculipalpis* and *stephensi* Several other species collected in small numbers were not dissected but retained for museum purposes The work of dissection and examination was carried on continuously almost daily, throughout the seven months of the investigation The weekly records presented are of specimens in which both salivary glands and mid gut were examined The material giving only partial information is not recorded

The species examined were first observed in village habitations on the following dates —

A fuliginosus	first week in March
A maculipalpis	second week in March
A culicifacies	second week in April
A stephensi	first week in May
A subpictus	last week of June

The meagre rainfall in this district doubtless influenced *mosquito production* Until the middle of July less than one inch of rainfall was recorded for any month The dissections recorded up to 1st July totalled 1,672 and up to 18th September, when the study was completed, 5 052 specimens of salivary glands and an equal number of stomachs were dissected and examined for the presence of plasmodia

The specimens were distributed numerically during the seven months as follows —

A stephensi	218	specimens	4 9	per cent
A maculipalpis	258	„	5 1	„
A fuliginosus	875	„	17 3	„
A subpictus	1,650	„	32 6	
A culicifacies	2,021	„	40 0	„

A total of 3 385 specimens had been examined up to 15th August without observing insect plasmodia On this date an infected *culicifacies*, which had been collected on 9th August, was detected In this specimen 2 oöcysts were found both exhibiting vestiges of malaria pigment and with a maximum size of 55 *microns*

The second infected mosquito, collected on 26th August, was observed with 71 oocysts measuring from 28 to 55 microns, with an average size of 46 microns They were mostly attached to the caudal portion of the mid gut There was no evidence of sporozoites in either of these two mosquitoes

The date of finding the next two infected mosquitoes was on 30th August, and a fifth specimen was obtained on 8th September These three were in advanced stages of infectivity with a moderate *invasion of gland sporozoites and only* ruptured capsules *of oocysts to mark the stomach infections*

Meteorological considerations —In an analysis of the records of humidity of the seven months of the investigation a striking correlation appears between the weekly mean humidity percentages and the appearance of plasmodia in the dissected mosquitoes The highest mean percentage of humidity occurred during the first week of August rising from 78 during the last two weeks in July to a mean of 96 86 which was maintained in slightly decreased amount to the second week of September The dates of appearances of infected mosquitoes namely, 9th August 26th August, 30th August, and 8th September, seems more than a fortuitous *occurrence*

This tends to confirm the early work of Bentley at Bombay and that of Gill in Lahore and should afford added impetus to a more critical investigation of meteorological factors influencing mosquito infectivity

Discussion —It is generally agreed that the mental and physical discomforts occasioned by the extremes of temperature are aggravated two fold in the presence of increased humidity And this no doubt is heightened by the human attractiveness for mosquitoes which appear now in such profusion Possibly factors of this nature are associated with susceptibility to symptoms of latent malarial fevers Then it is that these relapse cases, harbouring parasites which have attained sexual maturity, form such an important link in the perpetuation of malaria from season to season They are drawn out from the condition of obscurity in which they are lingering during the period of æstivation and converted from a relatively innocuous status of individuality to a more dangerous community status For it is now that we are dealing with a communicable disease The critical situation is provoked usually at this juncture by the maximum increase of insect carriers and the biological factors are at an optimum for the dissemination of new infections

It is obviously important to establish as a fact that there exists a well defined dormant period in the incidence of malarial fevers in India If it can be accurately gauged that a sharp line marks æstivation from activity then can the public health official determine that the origin of certain cases is probably of initial infection or definitely recurrences of a previous season Possibly this information might prove of practical benefit in recommending when persons free of the disease may with safety disregard or on the other hand should observe precautionary measures Particularly applicable would this be in the instance of military units on the march away from protected stations

This measure of security obtained in the proper interpretation of information acquired relative to the interval when mosquito carriers do not function must be accepted with limitations of specific knowledge of the locale involved One must emphasize that a general promulgation of this information is not intended that it must be tested from year to year in the same region and in different regions in the same year

Were it possible or desirable to apply remedial measures on an extensive scale in an endemic focus of circumscribed area the favourable time for selection would probably be when latent or residual malaria only were present and the possibility of re infection is at a negligible stage That period would correspond to the inactive stage of malaria dissemination marked by the impotence of sporogonic development in the mosquito host Suppressive measures when based on this principle, it is realized, would be applicable only in dealing with bodies of men held under suitable control These measures are well recognized and one need merely indicate the period when it would be most economical and feasible to apply them

To be sure, the accuracy of determination of initial infection must be gauged by the effort expended for negative evidence is evaluated by its mass One might be justified in the saving of time and energy, in dissecting large numbers of

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To be sure, the accuracy of determination of initial infection must be gauged by the effort expended for negative evidence is evaluated by its mass One might be justified in the saving of time and energy, in dissecting large numbers of

Anophelines, to ignore all of the weak or questionable carriers, in this instance, *Anopheles subpictus* In this investigation this species comprised nearly one third of the total number of specimens collected and examined However, one does not presume to accept the responsibility for this recommendation inasmuch as there looms the possibility of a species changing its habits from time to time *A subpictus* in these studies, did not appear in appreciable numbers until the first week in July, and one given to speculation might suggest that this species, in all its perversity may change its habits relative to seasonable appearance and associated malaria incidence

In common probably with most workers dissecting numbers of Anophelines, it was the practice to keep alive collected specimens of mosquitoes for a short time, in order to permit them to clear the last meal of blood For this purpose, blood engorged insects were kept in suitable glass jars with ends enclosed in cloth bobbinet and furnished with moisture and fruit juice There was observed, in the course of the investigation a decided seasonal difference in the clearing process of engorged mosquitoes During the hot dry months until the end of June, engorged specimens rarely cleared the alimentary tract under 3 to 5 days When the relative humidity increased, it was observed that this process was distinctly shortened It was found feasible to dissect specimens for gut examination in 1 to 3 days after capture

This phenomenon leads one to speculate on the increased potentialities of the insect during the favourable period of malaria transmission Probably in the humid environments in contact with the host, more meals of blood are extracted, a ready natural emetic being provided Consequently greater opportunities for infection are then afforded

Then if one would care to indulge more deeply in theory and seek the factors contributing to the successful parasitism of Anophelines, I should repeat Sir Patrick Manson's advice to 'follow the flagellum' For at this time, at the height of the rainy season nature presents the optimum conditions of temperature and humidity expressly favouring the ex flagellation in the mosquito essential to successful launching of the sporogonic cycle One may be pardoned in alluding to that simple dodge, of inducing fertilization of the gametocyte in blood drawn on a glass slide, by introducing the warmth and moisture of the breath to accelerate flagellation.

A NOTE ON SOME EXPERIMENTAL ATTEMPTS TO TRANSMIT MECHANICALLY MALARIA ORGANISMS THROUGH MOSQUITO BITING

BY

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In a short research on the question of the minimum dose required to produce malarial fever measured by the infective bites of mosquitoes the following results were obtained —

Three Anophelines infected by biting a gametocyte carrier 18 days previously were observed to convey the tertian malarial attack to three new hosts when applied by probing the skin for precisely 50 seconds 35 seconds and 15 seconds respectively

Impressed with the ease with which an Anopheline could transmit malaria infection through the normal biological channel, a diversion was afforded in an opportunity to test out the possibility of mosquitoes playing a rol in immediate transference of plasmodia

The only reference available in which allusion is made to a possible vector of malaria parasites in a mechanical mode besides the early work of Grassi is that of Sacharow quoted by Blacklock (1921) In this instance leeches were used to preserve infected blood while kept on ice for a period of four days One c c of blood from this source, when injected produced typical malignant tertian malaria

Recently observations reported by Falleroni (1926) in Italy anticipate in a measure the negative results of attempted transmission of plasmodia in a direct manner Falleroni in a study of the physiological processes associated with the parasitism of *Anopheles maculipennis* draws attention to two different suction processes correlated to the different uses of the liquid sucked by the mosquito The food consisting of blood and fruit and other vegetable juices are consigned to different compartments, the first to the stomach the latter to the œsophageal diverticula or food reservoirs

There is evidently no regurgitation the two food elements are isolated and digested separately The first is derived by a puncture process but the latter is not obtained through biting only through what the author distinguishes as a process of simple aspiration Blood passes at once into the stomach of the biting Anopheles and Falleroni, aided in his conclusion by the mutilation of various parts of the

mouth apparatus excluded the possibility of a direct transmission of malaria Experimental evidence is not given

When compared to mechanical insect conveyance of trypanosomiasis specifically that of *Trypanosoma evansi* the organism is quite easily carried on the fouled proboscis of the fly from horse to horse In studies made by the writer a single fly was observed to act as porter for sufficient organisms to cause the disease in the fresh host However when a fly was induced to bite as many as four alternate hosts successively and interrupted upon each application *within a minute of the* insertion of the proboscis and not permitted to complete its meal, the results showed that organisms were not conveyed beyond the first contact These experiments were conducted to ascertain the probability of protozoan disease being transmitted *successively* through the agency of an insect porter

The experiments presented in the following report were conducted in a United States Government hospital for nervous cases Here malaria therapy for general paralysis of the insane was administered and provision was made for ample material for the prosecution of these studies There were available at this time three suitable febrile cases of tertian malaria showing on examination numerous rings and moderate numbers of gametocytes and fourteen selected cases were assigned for experimental insect conveyance The mosquitoes employed were *Aedes thibaulti* and *Anopheles quadrimaculatus* The specimens of aedes were collected in the wild state, and the Anopheles were laboratory bred

The method used consisted of the rapid transfer from infected to clean host allowing the mosquito to draw blood for less than a minute from the malaria patient then engorge itself while on the second host By this mechanical application eight experiments were completed with the *Aedes* mosquitoes using from two to fifteen specimens on both hosts There were four trials made with *Anopheles quadrimaculatus* in which three and as many as 40 specimens were applied alternately without an appreciable interval to both hosts The mosquitoes were applied singly several at a time while held in small glass cylinders closed at the ends with cloth netting The twelve patients were kept under observation for three weeks to thirty days then dismissed and further tested for susceptibility by various methods of malaria parasite injections

Results

Forty five specimens of *Anopheles quadrimaculatus* survived for fifteen days following the attempted mechanical transmission These were applied over a three days' period to two clean hosts both of whom developed tertian malaria in sixteen to seventeen days following the last biting

Dissections of the fed mosquitoes demonstrated nine infected specimens all with *sporozoites in scant numbers and two harboured in addition* a small number of oocysts The specimens of aedes which were dissected were free of infection

In considering the practical application of mechanical dissemination there may be one possibility, however remote, in which this method might operate In

a fulminating malaria epidemic *especially in the absence of a large number of* suitable gametocyte reservoirs one might ascribe the seeming wild fire dissemination to the partial agency of swarms of insect hosts and by their attacks effect a *direct transfer of asexual parasites*

These studies are logically associated with an attempt to arrive at the minimum infective dose of material either contained in a hypodermic syringe or present in the *proboscis of an insect and it is to be appreciated that the present report is preli*minary to work being contemplated The scope of these studies would normally take account of measured quantities of infective material such as the accurate enumeration of blood parasites by the Sinton method As far as I am aware no exacting experimental evidence of this type has been offered but doubtless the impetus given to protozoal therapy of paresis through the inspiration of Wagner Jaureg James York and others will stimulate it

An early reference to probably the smallest dose of blood containing malaria organisms possible to convey successfully is cited by Bastianella and Bignami (1899) in experiments involving transfusion as 0 2 c c

Later Marchiafava and Bignami (1900) state that a subcutaneous injection of less than one drop will suffice They found that the transmission of the disease by injection of blood occurs whether blood is taken during the apyretic period or during a febrile paroxysm whether it contains young parasites or those in process of development

In the association of minute doses and insect porterage a few attempts were recorded in the present report To determine whether the proboscis of the mosquitoes used in these experiments still retained plasmodia following the interrupted bite examinations were made of the dissected mouth parts

Three such trials were made with the aedes and two with Anopheles With both species the head of the fed mosquito was snipped off and a saline suspension made of the macerated proboscis This was managed without the aid of an anaesthetic in some cases and in others chloroform was used

In the instances where the dissection was performed before the mosquito was permitted to resume the biting of the second host plasmodia were noted in stained material thus obtained In the instance of an Anopheles the first host was bitten for a timed period of two minutes an interruption of forty seconds ensued, the uninfected host bitten for fifty seconds immediately after which the *mosquito* was anesthetized and the head severed *Here no malaria* organisms were observed although blood elements were distinguished in the dissected proboscis

I have found that *the contents of the stomach* of a specimen of *Anopheles quadrimaculatus* when injected subcutaneously produced tertian malaria In this particular instance numerous ring forms of *Plasmodium vivax* were observed in the blood of the bitten host Parasite counts were not made It is presumed that unless a mosquito in biting regurgitates its stomach contents almost immediately into the abraded skin of its second host, it is not likely that infection

will result In this connection it has been determined that an average sized Anopheline may imbibe about 3 milligrams, an amount of blood equal to its body-weight

Another factor to account for the failure of mechanical transmission by the direct method of biting may be assumed as the rapid drying of blood infested plasmodia on the external surfaces of the exposed mouth parts For it has been *demonstrated that although a subcutaneous injection of as little* as one minim of blood suffices to produce malarial infection, the organisms are destroyed when blood swarming with them is left to dry at the temperature of the air for a very short time

The possibilities of another sort of mechanical process was investigated in connection with sporozoite infective mosquitoes In addition to the aim of prolonging the life of the caged mosquito by supplementing the blood diet with *fruit juices it was found possible* in this connection to recover sporozoites from infective Anophelines After the usual incubation period sterile dates were placed in the mosquito cages to effect the discharge of sporozoites This was associated with the insect's efforts to pierce through the skin of the date and suck its juices

Active sporozoites indistinguishable from gland parasites were recovered in suspensions made from under the surface of the probed fruit The longest time *that motile sporozoites were observed was fifteen hours following the removal of* the fruit from the mosquito cages It was feasible to inject this material in a bacteria free condition into a human host, though the organisms were apparently too few or did not survive to convey the infection

DISCUSSION

(*Continued from page* 639 —ED)

Dr J W Scharff (Straits Settlements) I am glad to have this opportunity to hark back to Dr Strickland's report on the good results that he has obtained in India by the application of the biological measures applicable to *Anopheles maculatus* I *refer to the method of allowing jungle to grow up in ravines It is, therefore,* with surprise that I find Col Gill advocating jungle cutting without reference to the larva that breeds in the localities where he advocates this measure It has been stated by Col James and by Col Gill that economic prosperity determines the reduction of malaria virulence but, under conditions that appertain in Malaya the reverse is the case, namely, that anti larval measures are the only ones which will raise economic standards It has been suggested that, in advocating anti larval measures we are out [illegible] ance with the times I do not think we should be accused of direct path opened up to us by Sir Malcolm Watson by years of patient research We try to carry on that method by constantly improving and perfecting the details and eagerly look for anything which might be better I am of opinion that the measures advocated by Col Gill are of great value, but the field worker must guard

against diffusion of effort When possible we want to hit our mosquito malaria-carriers a staggering blow such as can only be visualized by an attack on the larvæ

Some observations that I have made indicate that *Anopheles maculatus* once it has fed upon its human victim proceeds at once to earth crevices in the neighbourhood of its breeding place, hence the suggestion that people should kill the adults of this species at least is not likely to be successful To my mind the danger of declaring dis belief in the value of anti larval measures is that those who control the purse strings will withhold funds until we have all agreed on some universal policy This as far as Malaya is concerned, would be a great misfortune for the unfortunate sufferers in places that still remain malarial

Dr A L Hoops (Straits Settlements) I was particularly interested in Dr Strickland's paper yesterday because it brought to my mind the time when he stayed with me in the Unfederated Malay States of Kedah in 1917 and taught me the importance of preventing the development of *Anopheles maculatus* by depriving it of its breeding places At that time there was great activity in the planting world and many estates were being opened up in Kedah some of them in hilly land The European medical officers who would normally have been serving in Kedah were serving in the Great War and I was almost alone I was however able to warn our estate managers both European and Asiatic of the danger of felling ravines on the newly opening estates, and thus providing the dangerous *Anopheles maculatus* with a breeding-ground in the sunlit running water of the hill streams Some of the managers took the warning and by sacrificing the use of the few acres of their land where ravines were situated, their whole estate force remained free from malaria Others neglected the warning and I remember one large estate belonging to a wealthy syndicate of planters, where the whole estate was felled and the coolie lines were placed near a running hill stream The coolies invariably went down with malaria in a few weeks, and absconded One labour force after another had to be recruited for that estate at great expense Finally the coolie lines had to be erected on another site

I instance this to show the importance of letting *well* alone Malaria can to some extent be controlled without any expenditure simply by avoiding the location of breeding places

Mr R Senior White (Bengal) addressed the following questions to Col James

Did he control the humidity in his incubators? Was it constant?

He endeavoured to repeat the experiments of Col Christophers and Dr Puri by pipetting centrifuging and counting plankton organisms from an artificial rain pool Bacteria were not counted, but with regard to organisms visible in the unstained condition under a $\frac{1}{6}$" lens he found the maximum food concentration at about 8 mm depth on an average, which is out of the reach of an Anopheline larva

He asked Dr Strickland, what plants were grown on the Amlutia estate and how long did they take to make an effective cover?

Dr S L Sarkar (Bengal) In Hindu and Mohammedan times the towns were so planned that there were facilities for the system of flood and flush, which served as anti larval measures I have studied this in the ancient town of Gour, which was the ancient capital of Bengal in the Mohammedan times The drainage system of ancient

Gour was studied by the engineering branch of the Public Health Department at my request and a map of the drainage system has been published by me in the *Indian Medical Gazette* last year The site of the ancient town was so selected that it was situated between two flowing rivers viz, the Bhagirathi and the Mahananda and the drain were flushed by the floods of the rivers As every tank was connected with these drainage canals every one of them was flushed A part of the one became intensely malarious by the closure of some of these drainage channels The splenic index of this region became 81 per cent while in the portion which was flushed the splenic index was 18 per cent When the people of the locality cut down a bundh named the Lohagorah bundh by which a part of this non flooded area became flushed the splenic index became considerably reduced The details have been given in the article in the *Indian Medical Gazette* I have already mentioned

Dr C Strickland (Bengal) I am afraid I cannot inform Mr SeniorWhite of the botanical names of the jungle plants grown at Ambutia They were in most cases young saplings taken from jungles growing in the neighbourhood This would be the best procedure to adopt in most places I could doubtless get the plants identified for Mr SeniorWhite if he wishes it

Major H H King (Madras) Asked Col James to give details of the temperature and humidity conditions in his experiments on longevity in Anophelines He entered a plea for the prevention of the production of mosquito breeding places He mentioned a survey recently done by the King Institute in the Mopad area where irrigation works had produced an epidemic of malaria A river had been dammed to provide the water so that below the dam the river had become a series of pools freely breeding mosquitoes Further, on account of the free use of irrigation water for rice fields and a rise in the subsoil water the whole area was breeding Anophelines in large numbers The fact that the Public Health Department had not been consulted when this irrigation work was sanctioned was brought forward as an instance of the necessity for the conference passing a resolution to the effect that plans of engineering works like these should be submitted to the public health authorities before being sanctioned (*He was asked to draft such a resolution*)

Dr N Banerji (Bengal) I represent the Birnagar Palli Mandali which is a Malaria Control Association working at the town of Birnagar in the district of Nadia (Bengal) This Association was formed in October 1923 and it has within its scope the control of Anopheline mosquitoes and mass quininization Detailed records of work are being maintained by the Society and the analysis of data has been undertaken on approved statistical principles Much valuable information regarding the breeding and hibernation of mosquitoes has been collected during the last four years and new light has been thrown on the epidemiology of malaria After very careful observation we have come to the conclusion that the causes governing the epidemiology of malaria in the Nadia district and perhaps the whole of western Bengal are as follows —

(1) Uninterrupted breeding of Anopheline mosquitoes in tanks and other reservoirs of water during the winter and summer months

(2) Absence of any heavy and continuous downpour or storm during the early part of the monsoon which enables swarms of mosquitoes to pass uninjured from the

state of inactivation to one of activity, so that they have full scope to spread infection and to multiply

(3) Early monsoon specially preceded by the spring showers of April and May This results in an early break in hibernation followed by the onset of an epidemic in a few days A careful examination of the fever incidence curve for the non quinine population showed that almost each time after a shower the curve shot up True hibernation stops at the first showers preceding the monsoon The first break in hibernation is followed by periods of inactivity on the part of the adult mosquitoes as far as their biting propensity is concerned whenever there is a spell of dry weather for any length of time Each fresh shower brings about a break in this inactivation resulting in a greater incidence of the infection The reasons for arriving at this conclusion are fully stated in the Annual Report of the Society which has been published in the *Calcutta Medical Journal* for December 1927 a copy of which will be supplied to each member attending this conference in a day or two

The Director of Public Health Bengal takes a spleen census of the children at Birnagar almost every year The comparative splenic index prepared by him reveals the remarkable fact that the spleen rate fell from 79 per cent to 28 per cent in one year solely owing to quininization We have distinguished between the extent and intensity of malarial infection The total number of individuals suffering from fever at some period during the year indicates the extent of infection and the maximum number of persons suffering at any particular time indicates the intensity of infection at that period

I have been rather disappointed at some of the papers read at the meeting which discourage the use of anti larval measures when there is insufficiency of funds Our experience has been very different at Birnagar In a small area of 2¼ sq miles it is quite possible to combat malaria successfully by the use of anti larval and mass quininization methods with say, Rs 6 000 a year unless the conditions are very unfavourable At Birnagar, there has been a marked improvement in the health and sanitation of the place during the last four years of our work and part of it is due to anti larval measures as is apparent from the marked fall in the fever incidence curve even among the 'non quinine' population

In ten years if we continue our vigorous campaign we will be able to eradicate malaria from the place

Col A B Fry I M S (Bengal) said The importance of adult destruction in barracks was impressed on him by the great work done by the League of Nations and the Ministry of Health on the persistence of infection in and the long life of the female Anopheline In the Meerut district he inaugurated an anti 'adult campaign The method of catching was to use the soldiers Three men worked a barrack catching as many mosquitoes as possible by the use of their bare hands covered with soap suds The barracks were very easily cleared with good results both at Meerut and Dehra Dun He also wished to emphasize the value of these congresses in bringing about co-ordination of ideas It was now made clear that every locality had its own malarial problem and workers need no longer waste time in trying to convert to their own views others who were working under a totally different set of conditions

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Lieut Col J B Hanafin I M S (Punjab) Drew attention to the importance of action against adult mosquitoes as exemplified by the work done in Lahore cantonment (Mian Mir) during the years 1926 27 —

Anti larval methods of malarial control which have been tried for years in India have failed or met with only partial success Conditions here differ from those in Malaya where Sir Malcolm Watson has had such a brilliant success The anti larval methods energetically carried out by James and Christophers in Mian Mir in 1902 and 1903 with so little success can well be compared with the anti 'adult' measures carried out in 1926 and 1927 in the same cantonment (1) The infantry barracks were completely and effectively mosquito proofed by gauze wire in July 1926 (2) Systematic fumigation has since been done by vaporizing saponified cresol over braziers 4 to 6 ounces to each 1 000 cubic feet The results are given in the following two tables Table I shows the malarial incidence for proofed barracks (1926 27) as compared with the results obtained in former years when the barracks were unproofed Table II shows the incidence for proofed and unproofed barracks in the same station in the same year The infantry barracks only were proofed

TABLE I

INCIDENCE OF MALARIA IN PROOFED AND UNPROOFED BARRACKS FOR DIFFERENT YEARS (MALARIAL MONTHS ONLY)

British Infantry, Lahore Cantonment

1st August to 31st October each year

Year	Barracks	Average Strength	Malaria		Dengue, Sandfly, Influenza and P U O	
			Admissions	Ratio per 1 000	Admissions	Ratio per 1 000
1923	(Unproofed)	588	500	850 34	*Nil*	*Nil*
1924	( )	489	236	482 62	4	8 18
1925	(Napier Lines only) (Unproofed)	281	160	569 40	17	60 67
1926	(Napier Lines only) (Proofed)	302	55	182 12	*Nil*	*Nil*
1927	(„ „ „) („)	285	13	45 61	2	7 01

TABLE II

COMPARISON BETWEEN MOSQUITO PROOFED BARRACKS AND UNPROOFED BARRACKS IN THE SAME STATION AS REGARDS INCIDENCE OF MALARIA

LAHORE CANTONMENT

1st August to 31st October each year

All other fevers are also given

Year	British Infantry Battalion						Other British Units					
	Barracks	Average Strength	Malaria		Dengue Sandfly Influenza and P U O		Barracks	Average Strength	Malaria		Dengue Sandfly Influenza and P U O	
			Admissions	Ratio per 1 000	Admissions	Ratio per 1 000			Admissions	Ratio per 1 000	Admissions	Ratio per 1 000
19 3	Unproofed	588	500	850 34	Nil	Nil	(Unproofed)	35.	190	539 77	Nil	Nil
1924		489	236	482 6	4	8 18		309	95	307 44	4	8 18
1925		281	160	569 40	17	60 67		334	157	470 06	6	21 35
1926	Proofed	30?	55	182 1?	Nil	Nil		293	197	67. 35	1	3 31
1927	"	285	13	45 61	2	7 01		391	104	265 98	4	14 03

It is evident that the malarial incidence has been reduced to a sixth or a tenth of that obtaining in former years The figures are striking Figures for admission for other fevers which could be accidentally or intentionally confused with malaria viz, dengue sandfly fever influenza and unknown fevers are also attached These do not show any increase

Col James has shown us the domestic habits of the mosquito Although we have *failed to exterminate* it we can yet attack it in our houses or prevent it from getting in there I submit that for the present with the funds at our disposal an attack on the adult *mosquito by wire proofing bungalows and fumigation is the most effective method* we possess

Prof J W W Stephens (Great Britain) Mosquitoes with undeveloped eggs in October are I suppose, young mosquitoes from the last batch of the year They have a survival rate in the experiments of 66 per cent But mosquitoes in January also with undeveloped eggs have a survival rate of only 30 per cent I think it is possible that this difference is due to the fact that these mosquitoes are three months older than the previous lot Starting again in October with the value 0 per cent for 'developed eggs', we should have expected in March or April this value to have reached 100 per cent It may be however that only 25 per cent (or some such figure) of eggs ever reach full *development The figures are not easy of interpretation, for from March onwards we* are dealing with a mixed population of young middle aged and senile mosquitoes

Sir Malcolm Watson (Federated Malaya States) (Chairman) Thought everyone would agree with him that they had had a most profitable discussion They had had the subject presented in all its various aspects by speakers from different countries The speakers from Malaya and the United States had brought forward their long experience of anti larval measures in towns and villages and plantations, an experience extending *over 25 years Anti larval measures had been successful far beyond their expectation* and he asked those from other countries to give these a trial He was glad to see eye to eye with the League on one matter, the value of exchange of health officers He *thought that if some such exchange were possible with those engaged in* malarial control their differences would soon diminish If Col James had been in his place responsible for the welfare of some 3 000 coolies engaged on a large industrial undertaking constructing works costing £ 2 000 000, he would have adopted exactly the same measures as the speaker, namely, the preservation of jungle and the oiling of any water exposed to the sun The result had been entirely successful Instead of a labour force decimated by malaria the coolies were happy and healthy, and work was *not being delayed by ill health Financially this was of the greatest importance*

But when they turned from towns, certain villages, plantations large public and private undertakings to wide ranges of country, the position was entirely different There the destruction of larvæ was not necessarily the measure of choice Mr Iyengar had shown them how extensive areas in Bengal were so swampy that the people went about in boats, yet there was no malaria He, the speaker, had removed coolies from a high and dry site to the edge of a large swamp in 1910 as related under S C Estate in the 'Prevention of Malaria in the Federated Malaya States' *He had seen them quite* lately, the spleen rate was low and they were healthy Extensive areas of wet rice in many countries in Asia and America showed that rice fields need not be malarious If

they were, he was convinced that they could be made healthy The work of irrigation, accompanied by proper drainage, described by Col Gill in his paper was of the greatest importance By such means the health of the people would be improved their economic position improved, their food supply increased Even if for the moment it produced two Anopheles where before there were none research would in time open the way to the control of the mosquitoes without reducing the rice crop He had always insisted that, if the production of rice also produced malaria people must have the rice and the malaria No one would consent to starve himself to death in order to avoid malaria But the more they had learned of rice fields the less he feared them In Malaya research had been going on for some 15 years Strickland and Williamson had done much work Williamson in Malaya was in close touch with Senior White in India Both were exploring the conditions which controlled the presence of Anopheles in water and he had little doubt that this would lead to improved methods In some rural areas they must drain in others they could live without fear in a swamp In some places they must clear jungle Col Fry had told them that his life had been made a burden at one time by people insisting that jungle should be cleared away because jungle clearing was a success in Malaya In Malaya they had learned that the preservation of jungle was of great value in controlling malaria in certain places The Hon ble Dr Hoops Principal Medical Officer Straits Settlements had got a law passed prohibiting the clearing of jungle in certain areas, without the permission of the Health Officer

In the F M S the Malaria Advisory Board issued warning notices to the same effect It would thus be seen that while Malaya was united on the value of larva control and destruction in towns and other special areas as the method out in rural areas they were prepared to and did in fact, use any method which proved to be of value Each country must be studied in detail Any part found healthy would be a guide as to what they must do Local standards were therefore, of first importance

They had all one aim the control of malaria and he was sure that everyone would willingly accept and adopt any method of proved utility

A SUMMARY OF WHAT IS KNOWN OF THE SIGNIFICANCE OF THE SPLEEN RATE AND AVERAGE SIZE OF THE ENLARGED SPLEEN IN MALARIA

BY

BREVET COL S R CHRISTOPHERS, C I E, O B E, F R S, I M S,

Central Research Institute, Kasauli

THE spleen rate is now used in all parts of the world and has come to be the common method of measuring and mapping malaria No excuse is needed, therefore, for the investigation and critical consideration of the nature and significance of a test so extensively employed

I shall consider the matter here in two connections viz, (*a*) as the percentage of persons showing enlargement of the spleen, or the *spleen rate*, and (*b*) as the degree of enlargement of the spleen, or the *average enlarged spleen*

THE SPLEEN RATE

The spleen rate is the percentage of persons in a community showing palpable enlargement of the spleen It is now customary to restrict the term spleen rate to that in children, excluding infants and adolescents, and to refer to the rate among adults as the *adult spleen rate*

That in the absence of a high kala azar incidence the spleen rate if over one or two per cent may safely be ascribed to malaria is generally accepted But the exact relation of the spleen rate to the parasite rate (i e, to actual malarial infection) has been much discussed Various published data and general experience indicate a considerable correlation between the spleen rate and the parasite rate We cannot, however, expect this correlation to be exact Some children as emphasized by Ross who have not enlarged spleens show parasites in the blood on the other hand some with enlarged spleen give a negative result to blood examination

The general opinion as to the relation of the spleen rate to the parasite rate would appear to be that, when we examine a community, we find some individuals who have infections which have not given rise to an enlarged spleen, possibly because the infection has not lasted long enough or has not been sufficiently severe Other individuals are in such a stage that they show both infection (parasites) and the enlargement of the spleen associated with such infection, and still others have

recovered from the parasitæmia but still show enlargement of the spleen This may be called the *incidental theory of the spleen rate* It takes no cognizance of phenomena connected with the size of the spleen which phenomena we must now consider.

The Average Enlarged Spleen

The first to emphasize the value of a record of the size of the spleen in malarious communities was Ross Ross (1908) classed spleens as normal and as those roughly 3, 6 or 9 times the normal size In his Mauritius series these classes numbered respectively 19,711, 4,381, 3 479 and 2,566 What Ross termed the *average spleen* was obtained as follows —

19,711	at 1	19,711
4,381	„ 3	13 143
3,479	„ 6	20 874
2,566	, 9	23,094
30,137		76 822
	Mean	2 51 (times the normal)

Ross later (1910) omitted normal spleens from the calculation obtaining a figure which he termed the *average enlarged spleen* In the Mauritius series this was .—

4,381	at 3	13 143
3,479	„ 6	20 874
2,566	„ 9	23,094
10,426		57,111
	Mean	5 48 (times the normal)

It is not necessary that these averages should be calculated in size or weight The costal projection of the spleen, for example, can be measured and the average spleen, etc , calculated as so many finger breadths or centimetres projection

The average enlarged spleen on the whole is the more useful figure and will be employed here It gives the mean size, weight or projection, etc , of the enlarged spleen quite independently of the number of spleens enlarged

Christophers (1916) showed that the average enlarged spleen increased as the spleen rate increased In observations given by this author the value of the average enlarged spleen at low spleen rates was about 3 cms projection and an estimated weight of 140 grammes or about 3 times the normal With an increase in the spleen rate it rose to 6 or 7 cms projection and an estimated weight of 250 to 300 grammes or about 5 to 6 times the normal These values were very constant

The change in the average enlarged spleen is due to regular changes in the proportion of the different classes of spleen sizes as seen at different spleen rates The following table gives the figures for examination of spleens in the Punjab in 1909 reduced to percentages In the last column is given the average enlarged spleen calculated on these figures The numbers relating to the larger sized spleens are seen to increase as the spleen rate increases while the numbers of the smaller spleens decreases

Spleen rate	Percentage of enlarged spleens in each class (in cms costal margin projection)							Average enlarged spleen
	2	4	6	8	10	12	over 12	
30—40	51 1	33 6	11 4	2 3	1 5			3 38
40—50	42 2	22 9	25 7	2 8	4 1	1 9		4 16
50—60	41 8	28 6	17 6	6 6	3 3	2 2		4 16
60—70	38 9	31 6	20 5	3 1	5 2	0 4	0 4	4 14
70—80	31 2	32 9	19 2	8 4	6 2	1 2	1 0	4 67
80—90	28 2	33 3	20 2	8 6	5 5	2 9	1 3	4 88
90	23 1	34 2	20 0	11 8	6 5	3 7	1 0	5 21

Shown graphically the nature of the effect is clear from Fig 1 Here the frequencies for a high and a low spleen rate are depicted and a vertical line in the

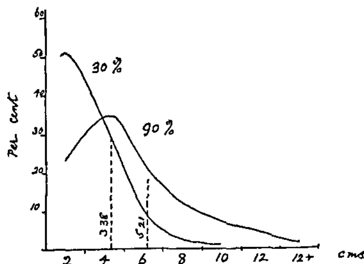


Fig 1

usual manner indicates the mean in each case This mean is obviously the average enlarged spleen The average enlarged spleen then is a convenient method of indicating a particular type of frequency

Below certain spleen rates however the frequency does not change and there is therefore no reduction in the average enlarged spleen If only a few children in many hundreds have an enlarged spleen yet when one has collected a sufficient number to get a reliable figure the average enlarged spleen is still about 3 4 costal margin projection

The peculiar shift of the frequencies with its corresponding effect on the average enlarged spleen the fact that the average enlarged spleen has a definite minimal value and that the values for the average spleen for a given spleen rate are remarkably constant even in different countries constitutes at present the riddle of the spleen rate

Why for instance should the spleen on the average never be below a certain size and why because more people have enlarged spleens should these spleens on the average be larger ? To explain this I put forward some years ago the suggestion that a single untreated infection in a child causes on the average a certain enlargement of the spleen which is thus a kind of unit or as I called it a *splen* Further I supposed that a superposed infection increased this enlargement If this were so then by the well known laws of chance distribution some people would get more superposed infections than others and so one might get the effect in question

If 100 infections are distributed by chance among 100 people this will not mean that each person gets an infection but that the chances are that 37 people will escape infection 37 will get 1 infection 18 will get 2 and 6 will get 3 infections If 200 infections are scattered the numbers respectively of 0 and 1 to 6 infections will be 13 27 27 18 9 4 and 1 These figures therefore have rather a resemblance to the proportions of different sizes of spleen seen in malarious communities This may be called the *overlapping infection theory of the spleen rate* It endeavours to explain the phenomena of the size of the spleen as well as merely the spleen rate

Measurement of the Spleen

Before proceeding further it is desirable to say something about methods of measuring the spleen and to give a brief idea of the procedure in this respect now being adopted in India in studying the spleen rate

Both Ross and Christophers desired to get at the weight (or what is the same thing the size) of the average spleen But it is undesirable to be dealing with estimates if we can find any way of recording actual measurements If we really want the weight it is better to measure the projection of each spleen ascertain the average projection and estimate what the weight of a spleen of just that degree of projection would be For the present however we need not deal with the weight which can be estimated later at any time from our actual measured observations on projection etc

Measuring the projection of the spleen in finger breadths or centimetres beyond the costal margin (1 finger breadth=2 centimetres) is a natural and easily understood procedure It is only a modification of the same principle if we measure the distance of the apex of the spleen i e its most prominent point

from the umbilicus or from the middle line of the body These different measurements are much less confusing now we know something of the proportions of the child's abdomen It is obvious that the enlarged spleen extends to a certain part of the abdominal wall and if we mark this on a chart of the abdomen drawn to measurement, we can measure any number of lines—they will all go to the same point

A difficulty which has always been present until recently, and which is perhaps to some extent still existent has been the fact that in measuring the spleen in children of different ages, and therefore of different sizes the absolute measurements so taken are not proportionately correct A spleen of 6 inches might be an enormous spleen in an infant, but not nearly so proportionately large in an adult For this difficulty correction tables based on average body measurements for different sizes of child can be used and all actual measurements reduced to those for a standard child, i e , the mean child 2 to 10 or one of sitting height 60 cms I give such a correction table for Indian children Macdonald (1926) has made a similar table for African children

CORRECTION TABLE FOR SPLEEN MEASUREMENTS

Showing correction for measurements of spleen or other abdominal measurements by the sitting height, nipple umbilicus line or age (recession 0 8)

H	N U	Age	Measurement in centimetres																		
			2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
0		2	3	4	5	7	8	10	11	12	14	15	16	18	19	20	22	23	25	26	2
1			3	4	5	7	8	9	11	12	13	15	16	17	19	20	21	23	24	25	2
2			3	4	5	7	8	9	11	12	13	14	16	17	18	20	21	22	24	25	26
13			3	4	5	6	8	9	10	12	13	14	16	17	18	19	21	22	23	25	26
14	16		3	4	5	6	8	9	10	11	13	14	15	17	18	19	20	22	23	24	2
15			2	4	5	6	7	9	10	11	12	14	15	16	17	19	20	21	22	24	2
16		3	2	4	5	6	7	9	10	11	12	14	15	16	17	18	20	21	22	23	25
17	17		2	4	5	6	7	8	10	11	12	13	15	16	17	18	19	21	22	23	24
18			2	4	5	6	7	8	10	11	12	13	14	15	17	18	19	20	21	23	24
19			2	4	5	6	7	8	9	11	12	13	14	15	16	18	19	20	21	22	23
50	18	4	2	3	5	6	7	8	9	10	12	13	14	15	16	17	18	20	21	22	23
51			2	3	5	6	7	8	9	10	11	12	14	15	16	17	18	19	20	22	23
52			2	3	5	6	7	8	9	10	11	11	23	15	16	17	18	19	20	21	27
53	19		2	3	4	6	7	8	9	10	11	12	13	14	15	17	18	19	20	27	22
54		5	2	3	4	5	7	8	9	10	11	12	13	14	15	16	17	18	20	21	22

CORRECTION TABLE FOR SPLEEN MEASUREMENTS—*concld*

S H	N U	Age	Measurement in centimetres																		
			2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
55			2	3	4	5	6	7	9	10	11	12	13	14	15	16	17	18	19	20	21
56	20		2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19	20	21
57			2	3	4	5	6	7	8	9	10	11	12	14	15	16	17	18	19	20	21
58		6	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	20	21
59			2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
60	21		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
61		7	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
62			2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	19
63	22	8	2	3	4	5	6	7	8	9	10	11	12	13	14	15	15	16	17	18	19
64			2	3	4	5	6	7	8	9	9	10	11	12	13	14	15	16	17	18	19
65		9	2	3	4	5	6	7	7	8	9	10	11	12	13	14	15	16	17	18	19
66	23		2	3	4	5	6	6	7	8	9	10	11	12	13	14	15	16	17	18	19
67		10	2	3	4	5	5	6	7	8	9	10	11	12	13	14	15	16	16	17	18
68			2	3	4	5	5	6	7	8	9	10	11	12	13	14	14	15	16	17	18
69	24	11	2	3	4	4	5	6	7	8	9	10	11	12	12	13	14	15	16	17	18
70			2	3	4	4	5	6	7	8	9	10	11	12	13	14	14	15	16	17	18
71		12	2	3	3	4	5	6	7	8	9	10	10	11	12	13	14	15	16	17	17
72	25	13	2	3	3	4	5	6	7	8	9	9	10	11	12	13	14	15	16	16	17
73			2	3	3	4	5	6	7	8	9	9	10	11	12	13	14	14	15	16	17
74		14	2	3	3	4	5	6	7	8	8	9	10	11	12	13	13	14	15	16	17
75	26	15	2	2	3	4	5	6	7	7	8	9	10	11	12	12	13	14	15	16	17
76		16	2	2	3	4	5	6	7	7	8	9	10	11	12	12	13	14	15	16	16
77			2	2	3	4	5	6	7	7	8	9	10	11	11	12	13	14	15	15	16
78		17–20	2	2	3	4	5	6	6	7	8	9	10	10	11	12	13	14	15	15	16
79	27		2	2	3	4	5	6	6	7	8	9	10	10	11	12	13	14	14	15	16
80			2	2	3	4	5	6	6	7	8	9	9	10	11	12	13	13	14	15	16

Note—Measurements of 1 cm are unchanged

Note—Readings for correction by nipple umbilical line or age should be taken along the line opposite the figures in the columns referring to these

S H = Sitting height N U = Nipple umbilical line

The observed measurements are those given in the top line of figures, the corrected values are in columns below these

The advantages of correction are that by reducing all measurements to a standard scale they can be dealt with *very readily and completely* whilst otherwise no use can be made of the data at all The objection to such procedure is that the body measurements used are variable The question at issue is not whether such points are fixed a position no one could take up but whether they are *approximately enough fixed to enable their mean position to be used* with useful results The whole procedure is an approximation and all that can be said for measured values is that without being mathematically free from error they are better than unmeasured ones and loose estimates

Oudendal (1925) has made careful measurements of anatomical points on the abdomen etc and the position and shape of the spleen post mortem His results show considerable variation in the position of the abdominal landmarks and in the shape and position of spleens of the same weight etc Whilst indicating the extent of variation and possibility of error even in detecting let alone measuring the enlarged spleen I do not think Oudendal s results must be taken as quite negativing the use of correction and efforts to measure the spleen with as much precision as possible In the first place Oudendal s data deal chiefly with adults where not only are the surface landmarks likely to be more variable than in children but the spleen itself is I have reason to believe likely to show more abnormalities of shape etc Moreover it is in children only that correction seems necessary and *it is with children that we are mostly concerned* in studying the spleen rate Oudendal I may mention was especially struck with the fact that in boys the degree of variation was much less noticeable than in adults

Further it is throughout with biometrical *means* that we are dealing From Oudendal s charts of boys showing variation in the position of the nipple etc it is very obvious that there is a definite mean position and that the variation from this is moderate

Perhaps most important of all from the practical point of view is the fact that the amount of correction involved in errors due to variation is not very great Suppose we correct a spleen measurement of 8 cms , in a child with a nipple umbilicus measurement of 16 cms (i e the smallest likely to be dealt with) the correction makes the measurement 10 cms If now there was a variation in some individual in the nipple umbilicus line even of plus 3 cms which is a very considerable variation this would only make the corrected value 9 in place of 10 Lastly when we are correcting for a toddler of 2 or 3 as compared with say a boy of 10 which is the real object of correction the correction is likely to be considerable and so the error due to variation less important

Everything considered and keeping a due sense of proportion as regards the degree of accuracy we hope *to get and making no claim whatever that* abdominal points are fixed there would not seem sufficient reason to abandon all idea of measurement or correction

By reducing all measurements to those for a standard child, considerable advantages are gained including the ability to compare data on a standard abdominal chart For a description and the uses of such a chart I must refer you to the original papers * dealing with this subject The figures on the screen will explain, I think, what is meant sufficiently

It is best not to use any single linear measurement to denote the projection of the spleen, but much more effective to fix the position of the apex or most prominent point of the spleen by triangulation For this two measurements are taken (a) the distance of the apex from the umbilicus and (b) the distance of the same point from the middle line of the body The method will be clear from the following diagram (Fig 2) At the same time the nipple umbilicus measurement is taken to enable correction of these measurements to be made

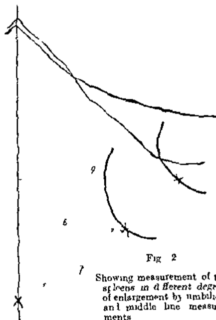


Fig 2

Showing measurement of two spleens in different degrees of enlargement by umbilicus and middle line measurements

for size of child In such spleen measurements a suitable sign, plus or minus, is used to express the quadrant of the abdomen in which the apex is situated i e, plus above and minus below the umbilicus and plus to the left and minus to the right of the mid line of the body

The mean position of the apex is obtained by taking the mean of each of the two measurements we have used in triangulation These two means themselves

* Christophers and Khazan Chand (1926), Christophers (1926), Macdonald (1926), Covell (1926)

fix a point which is the mean position of all the apices * Thus we write down in series our observations with the measurements in two columns, two further columns

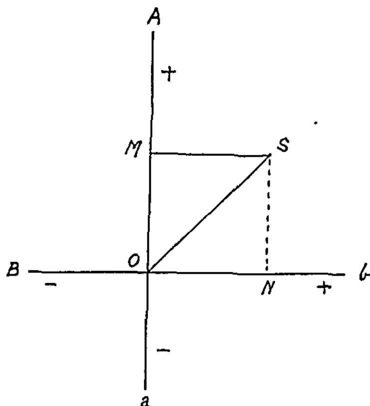


Fig 3

are made of these measurements corrected for size of child These two latter columns are added up and the sum divided in each case by the number of

* *Note* —This is not an absolutely correct method of calculating the mean position of the apex, but *an absolutely correct determination can be made at the cost of a little more trouble in calculation.* In a paper shortly to be published by Col McCombie Young (1928) the means have been calculated in this correct way and this author describes the method which I suggested to him Very briefly the projection of the two measurements are taken on two axes at right angles passing through the umbilicus as zero point The measurement to the mid line of the body requires no alteration, its projection is the same as the original measurement The projection of the measurement from the apex to the umbilicus must on the other hand be obtained on ordinary Euclidian principles by squaring this *measurement,* subtracting the square of the mid line measurement and taking the square root of the difference It is merely a matter of ascertaining one unknown side of a right angle triangle from two known sides What is meant will be clear from the figure where Aa is a vertical axis (mid line of body) [illegible] SO and SM are [illegible] to [illegible] ing [illegible] of [illegible] ion

for the mean apex

observations This gives two mean values which indicate the position of the mean apex

We fix then the position of the apices of all our spleens in relation to the abdominal superficies and we take the mean position for all these points which is the position of the apex of the average enlarged spleen for the community itself a point

We have now the approximate position on the abdomen of the mean spleen apex for any given community and we can compare the position of this point with that for any other community either by actually plotting the positions on the chart or recording the numerical values which fix the points On a standard abdominal chart as prepared with ruled lines for convenience in roughly plotting points I have marked with crosses the mean apex for two communities one with a larger mean spleen than the other [*not reproduced* See *Christophers* (1921)]

Position of the Apex of the Average Enlarged Spleen in Different Communities

The rather remarkable fact is disclosed by such methods as I have indicated that the position of the mean apex no matter what the spleen rate may be is usually so situated that it is within an area on the abdomen which might be covered by a crown piece almost by a rupee The position of the mean apex is not however quite identical in all cases and its variations within certain narrow limits appear to be significant i e they appear to be due to differences in the conditions relative to malaria

As the spleen enlarges the apex passes along a line from about the region of the 9th costal cartilage to the umbilicus and thence more transversely across the abdomen The distance from the costal margin where the spleen first makes its appearance with slight enlargement to the umbilicus is in the standard child about 13 cms The great bulk of the apices lie always on or near this line and the average apex is always on or near the line at some point between 0 and 13 cms 0 being at the umbilicus and 13 at the costal arch Indeed it lies between much more restricted limits than this viz so far as our observations go at present between 7 and 10 cms on this line

You will remember in a table giving some spleen measurements in costal margin projection that the average enlarged spleen as measured in this projection changed with increasing spleen rate from 3 38 to 5 21 and that I said that it might possibly rise to 6 or 7 but never in my experience higher This you will see is the same 3 cms or so which is all the variation we get in this value in different communities measured by triangulation It confirms the general fact that the shift of the mean spleen is at most a very small quantity in spite of impressions one is apt to get to the contrary

If we wish to study the frequency of which the mean spleen value is only so to speak the indicator we erect on a base line representing the costo umbilical line and its extension beyond the umbilicus a frequency polygon using the number

of spleens that are 0 1 2 3 etc cms *from the umbilicus* I show such a frequency polygon worked out in this way (Fig 4)

You will realise I think the importance of studying such frequencies and will readily see that it is the frequency of different sized spleens in various communities that is really the fundamental question at issue when we speak of the average enlarged spleen So long as the frequencies are of the same character the mean or average enlarged spleen serves as a sufficient index But if the *frequencies are*

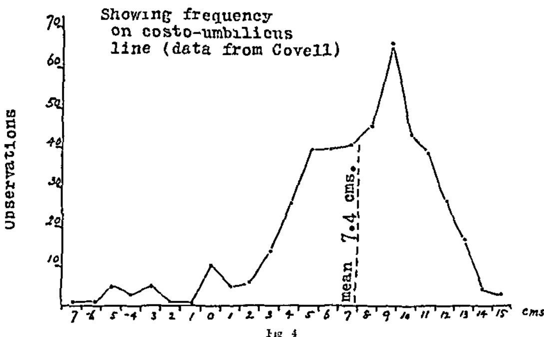


Fig 4

of different characters as you will see they are when I show you some more curves the mere consideration of the means may be inadequate or misleading Two different frequencies may for instance give the same mean

Ordinarily however the frequency has to be rather roughly determined and the mean becomes a good general guide to its nature I show two frequencies drawn on the same base line one with a mean of about 10 cms and one with a larger mean spleen viz about 7 cms (Fig 5) *

Whenever now in India suitable opportunities occur studying spleen rates are being studied in this way I may refer you to my own observations in Singhbhum etc and to an excellent study by Covell of the spleens in the Andamans as also to interesting studies on the spleen rate in Coorg by McCombie Young and Baily now in the press

* In these and subsequent measurements the smaller the apex umbilicus measurement of course *the larger the spleen*

So far observations on these lines are too few to enable final generalizations to be made but some tentative conclusions which still need confirmation may be mentioned

That in the tropics in malarious localities the children though not obviously suffering from malaria nevertheless are to a large extent infected is now well known This endemic condition has been studied by a number of observers and especially by Schuffner (1919) whose very thorough account of endemic malaria in the Dutch East Indies is one of the most important constructive contributions to our knowledge of the nature of endemic malaria Schuffner as others found the parasite infestation greatest in the early years of life becoming much reduced after the age of 3 or so The spleen rate however remains scarcely altered

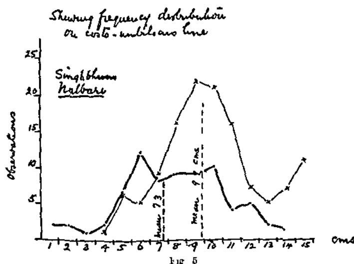


Fig 5

throughout the whole of childhood Christophers (1926) showed that the children in a hyper endemic community first pass through a stage of *acute parasitic infestation* lasting about 2 years with a very high average parasite value per c mm and that there then supervenes and continues through the rest of childhood a condition of *immune infestation* in which though the parasite rate for the community is still equally high the numerical value of infections is altogether different being very much smaller He showed also which is the important point in this connection that enlargement of the spleen beyond a certain moderate degree was associated especially with the latter period Hence the reason why with spleens of a certain size as many have found the bigger the spleen the more difficult as a rule to demonstrate parasites

This is a very different state of affairs to the conception of the incidental theory of the spleen rate where the enlarged spleen is the temporary concomitant or after math of discrete infections In these high spleen rates the whole life of the

child is involved and a large sized spleen is not so much determined by temporary infection as associated with a continuous state of infection plus immunity It would seem too that it is not overlap in the simple *form* I have *expressed it that* causes the shift of the average enlarged spleen It is not acute infections overlapping but some new state which appears to have supervened which accounts for the larger classes of spleen

Covell (1927) dealing with the spleen rate in adults in the Andamans found the ordinary resident convict to have an average enlarged spleen comparable to that of the children in the ordinary endemic state of the island i e it was about 7 cms umbilical measurement But in a community recently brought into a highly malarious locality and prostrated with malaria the spleen rate was equally high but the average enlarged spleen was about 10 cms measurement only

McCombie Young and Baily dealing with the average enlarged spleen in Coorg where the children were mainly born on the spot found what appeared to be an age period (7 to 8) when the size of the spleen was greater than at any other nearly all the largest sized spleens occurring at this time In actual practice the community is often not purely indigenous in the sense that all the children are born locally and so to speak start the malaria race level and this would ordinarily make it more difficult for us to see effects such as the above

It looks however as though there might be besides the normal spleen (*a*) two kinds of enlarged spleen (*b*) the spleen of acute malaria and (*c*) the spleen of immune malaria and that each of these spleens is biometrically distinct, i e with its own mean size and frequency (*b*) Can be envisaged as ranged in frequency towards the costal margin end of our base line with its mean about say 10 cms and (*c*) with *a range more towards the umbilicus end of the line with its mean say at* f cms Is it this which accounts for the riddle of the spleen rate ? It would appear possible that it is

Suppose the spleen rate is 30 per cent only It is doubtful if infection here would give any high degree of immunity Such children as have spleens are likely to be suffering then from acute infestation if not merely from incidental attacks of malaria and the average enlarged spleen will be low but not too low It will in fact be one splen the average enlargement from a single infection Reduction in the spleen rate will not affect the average enlarged spleen value Increase the spleen rate however and we get a greater proporton of children with immunity and developing the larger sized spleens With very high spleen rates we are dealing wholly with the spleen of immune infestation since the very young children the only ones with acute infestation are excluded when we take the spleen rate and all the others have reached the immune period

In the accompanying schema (Fig 6) I have put this theory into graphical form In the figure three *conditions are shown (1) a low spleen rate where immunity* has not entered into the picture and where the frequency and average enlarged spleen are wholly those of spleen (*b*) (2) a medium spleen rate where the two kinds of spleen (*b*) and (*c*) occur in various proportion and (3) a very high spleen rate where

the children are all in the immune infestation period and the frequency and mean is purely that of spleen (c) This view of the nature of the spleen rate recognizes

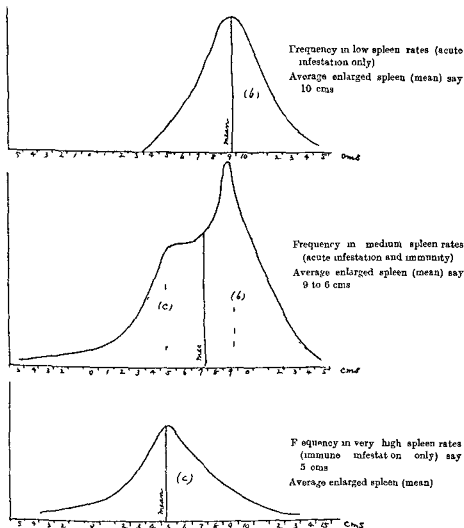


Fig 6

Schema to explain nature of alterations in the average enlarged spleen in malaria

The curves are illustrative only but resemble actual curves obtained in nature In the second case the unbroken line is the curve given by compounding the two dotted curves (*compare with fig 4*)

the incidental theory for low spleen rates but hypothecates for high spleen rates an entirely different nature whilst medium spleen rates according to this view are a mixture of these two states

The size of the spleen if this view be correct will still *usually* appear as a function of the spleen rate because the more intense the malaria (*provided it is sufficiently permanent*) the more children will have spleens and the more the large immunity spleens will come in But if the malaria is not static there may be a high spleen rate with a low average spleen

Macdonald dealing with the enlarged spleen in children in Freetown distinguishes in the town an endemic (low spleen rate) and a hyper endemic (high spleen rate) area In the former the average spleen was 10 5 and in the latter 8 1 cms from the umbilicus

Sergent Parrot Foley and Catenet (1927) make use of a splenometric index which is obtained by multiplying the spleen rate by the average enlarged spleen in finger breadths They refer to variations in the value of the average enlarged spleen with the same spleen rate etc With a spleen rate of 80 was found an average enlarged spleen of 1 9 i e about 3 8 cms costal margin projection or what would be about 10 cms in our nomenclature and with a spleen rate of 50 an average enlarged spleen of 2 3 or about 9 in our nomenclature With a spleen rate of 50 9 was a splenometric index of 213, i e an average enlarged spleen of 213/50 9 or 4 2 finger breadths which might be perhaps 6 on our scale

These results are not only in accord with the theory I have outlined but are made understandable by it

Further it is very noticeable how *closely these results follow measurements* in India In the case of Macdonald who was using the same technique as ourselves, the measurements for West African negro children are practically identical with *those for Indian children In the value of the average spleen we are dealing there*fore with something which is of world wide application and not something of merely local or incidental interest

The Spleen Rate as a Measure of Malaria

That the spleen rate is a measure of malarial intensity goes almost without saying *But what the exact value of the percentage figure may be* and what is meant by intensity is not so clear

In a continuously highly malarious community there are probably adjust*ments tending to keep* malaria more or less static Increase the infection and this will lower the age of the onset of immune infestation But in the stage of immune infestation gamete output is small By increasing infection therefore the number of acute infestations and therefore the number of effective carriers is reduced If infection is lowered there would be a tendency to postpone immune infestation and increase the number of effective carriers Hence some sort of a balance must tend to be struck

If infection is below a certain amount or is only temporary in character, it will not result in immune infestation and no balance due to this cause will result

Here then we have a sharp division on the one side what we have hitherto called hyper endemicity and on the other a more incidental type of malaria which we might call endemic merely Endemicity might be said to commence as soon as malaria is present Hyper endemicity might be said to begin when the frequency of infection is maintained at such a degree that some individuals at least are brought into the immune infestation state

How far the spleen rate measures the balance struck in hyper endemicity we cannot say But since the number of persons with immune period spleens seems to indicate the intensity the number of such individuals would become the measure of hyper endemicity Hence the measure of hyper endemicity would be the average enlarged spleen

When the infection rate is low or so long as there is no introduction of immune infestation the spleen tumefactions will be the result of discrete infections with at most such overlap as is due to chance distribution The spleen rate should therefore be a very good indicator of the frequency of such infections and thus in low spleen rates be a good measure of malarial intensity

With a very low spleen rate the average enlarged spleen should be low whether conditions are permanent and static or temporary and changing With medium spleen rates the average enlarged spleen value may give an indication of the relative degree of staticity of the infection With high spleen rates we may find a low average spleen or a high If low the high prevalence of malaria has only just started or it is temporary (seasonal)

It is premature perhaps to elaborate what is merely the view of the moment and liable to be changed by further observation I have given these considerations however chiefly to show the implications of the infection immunity theory of the spleen rate and to make the scope of this theory more intelligible Most of what I have said above is an explanation of the spleen rate as a whole from the point of view of such a theory supposing it to be substantiated There would of course be many other details The important thing however is that more careful work of the kind I have indicated should be done on the spleen rate Anatomical work on the spleen especially on the child s spleen is needed as also observations on children with enlarged spleen carried out over considerable periods and of course such observations must deal with both high and low spleen rates Field observations are essential but for useful work on the spleen such data must be of the kind that can be adequately studied and compared with other observers results and for this it would seem that the technique now being employed by us is eminently adapted Combined with Sinton s method of measuring parasitæmia it appears to bring the study of malaria conditions to a fairly satisfactory state as regards the collection of essential information Though perhaps demanding some expertness to carry out both accurate splenometry and measured parasite counts are perfectly practicable in the field and are really essential to the study of malaria in such conditions

REFERENCES.

CHRISTOPHERS, S R (1916) . . The spleen rate and other splenic indices, etc *Ind Jour Med Res*, Vol II, No 4, p 823

Idem (1924) . . The shape and position of the palpable portion of the enlarged spleen in children *Ibid*, Vol XI, No 4 p 1081

Idem (1924) .. The frequency distribution of measurements of the enlarged spleen in a malarious child community *Ibid*, p 1245

Idem (1924) . The mechanism of immunity against malaria in communities living under hyper endemic conditions *Ibid* Vol XII, No 2, p 273

Idem with KHAZAN CHAND (1924) The measurement in centimetres of the enlarged spleen in children, etc *Ibid*, Vol XI, No 4, p 1065

COVELL, G and BAILY, J D (1927) . Observations on malaria in the Andamans with special reference to the enlarged spleen in adults *Ibid*, Vol XV, No 2, p 300

McCOMBIE YOUNG T C, and BAILY, J D (1928) Malaria in Coorg *Ibid*, No 3

MACDONALD, G (1926) . Malaria in the children of Freetown, Sierra Leone *Ann Trop Med and Parasit*, Vol XX, No 3 p 239

OUDENDAL, A J F (1926) Enquiry into spleen palpation, based on the weight situation, shape and dimensions of the enlarged spleen in post mortem *Transactions of the Sixth Congress F E A T M* (Tokyo), Vol 2, p 235

ROSS, R (1908) . 'Report on the prevention of malaria in Mauritius' London

Idem (1910) 'The prevention of malaria' London

SCHUFFNER, W (1919) . Two subjects from the epidemiology of malaria *Meded v d Burg Geneesk d in Nederlandsch Indie* Deel IX, p 1.

IMMUNITY TO MALARIA

BY

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In my paper published in the *Indian Medical Gazette* of September 1913, on Malaria at Arambagh, a very unhealthy town in the Hooghly District I was able to show on the result of spleen census of children that though there was no difference in the intensity of malaria in the different parts of the town the members of some Hindu sub castes scattered through the town who were residing for a very long time showed less malarial infection comparatively than the other Hindu sub castes who were new comers Next when I was transferred to Nadia District I published a paper on 'Some Studies in Malaria in Nadia District which was published in the *Indian Medical Gazette* of April 1916 At the time of writing this paper it came to my notice that repeated infections during early life leave a very pronounced resistance against malaria I found the true significance of this development of immunity to malaria when I went to my next station Chittagong Hill Tracts, as civil surgeon There I found a definite law about splenic enlargement amongst the children of hill tribes which is not found anywhere else in Bengal This has been described by me in a paper entitled— 'A Peculiarity in the Spleen Rate as observed in the District of Chittagong Hill Tracts,' and published in the *Indian Journal of Medical Research* April 1921

The matter may be stated briefly thus Splenic enlargement was found to be very common amongst the children of the hill tribes, but practically absent amongst the adult population To ascertain the nature of splenic enlargement in children, the spleen census of a very large number of children of different ages from under one year of age to 12 years of age was determined and tabulated according to the age When a curve is drawn from this table showing the percentage of spleen for each year as ordinate and respective years of age as abscissa from a census for over two thousand children a very regular curve was obtained The curve after rising somewhat to the age of three years, shows a very regular and steady fall to the age of twelve, the fall from the seventh to the twelfth year being somewhat more abrupt than that from the third to the sixth year The following Chart is a copy of the curve taken from the above paper

This gradual diminution of the spleen rate of the hill children with advancing age can only be explained on the theory of development of immunity to malaria

Phenomena like this have been observed in other places by very distinguished observers though not in so definite a form as has been observed by me in the Chittagong Hill Tracts



Sir Ronald Ross, Christophers and others have observed that the splenic infection of children of comparatively advanced age is less than those of very young children

In such a malaria stricken region as the West Coast of Africa the death rate in residents of more than four years' standing is less than in previous years but this may be due to survival of more resistant immigrants But there can be little doubt that malaria in the Negro is a much less serious condition than in the European Koch from his observations in New Guinea attributes this to the infection of the native children leading to the development of immunity in the adult community Koch states that, while an immunity appears to exist in the native adults in malarial districts this is only true of those born in the locality, natives coming from the neighbouring non malarial districts into the malarial being liable to contract the disease It will be seen that these remarks are the very opposite to the facts collected regarding the Chittagong Hill Tracts area

The researches of Lieut Col S P James in connection with the malarial treatment of general paralytics have given us new conceptions in malaria I may

try to examine the phenomenon of immunity to malaria as noticed by me in the Chittagong Hill Tracts area in the light of these new researches The original book of Lieut Col James not being available to me I make a few quotations regarding his researches from the *Indian Medical Gazette* September 1926

Some of the mental patients whom Colonel James treated failed to show any thing more than a slight rise of temperature not associated with any clinical symptoms Now this was the usual type of fever noticed by me amongst the elderly children who had either benign tertian or malignant tertian parasites in their blood I have scarcely ever seen an adult belonging to the hill tribes suffering from an attack of malarial fever

Colonel James has found that the textbook description of benign tertian malaria is inaccurate There are differences between (a) Primary Malaria i e the benign tertian malaria following as a first attack after infection from the mosquito the patient never previously having had malaria in his life and (b) relapses in such persons after a first quinine treatment or after spontaneous recovery from the first attack

In the primary attack after the period of incubation after about 14 days the patient develops what Colonel James terms the initial stage of the attack which lasts from 2 to 5 days This begins as a gradually increasing irregular fever but towards the end of this stage is always intermittent

The initial stage is followed by the developed stage In 80 to 90 per cent of cases this is not a fever with tertian periodicity but a quotidian fever There is a rigor every day and this is true whether the patient has become infected by the bite of only one mosquito upon a single occasion or by the bites of many mosquitoes on several dates during the incubation period The developed stage lasts for ten days or often for longer

The type of fever now changes to the terminal stage and the temperature chart changes from a quotidian to a tertian fever with a rigor every 48 hours By degrees the patient recovers from the attack the symptoms diminish in severity and spontaneous (clinical) cure sets in

Such is the course of primary benign tertian malaria in the untreated subject and it is not described in any textbook If a primary attack runs a clear tertian course from the commencement it is worth while enquiring into the previous malarial history of the patient

Now in the malarial fever of the Chittagong Hill Tracts which was found solely confined to the child population amongst the hill tribes there were points which puzzled me very much at that time for which I now find explanations from the researches of Colonel James

The result of blood examination showed as a rule the presence of malignant tertian parasites Benign tertian parasites were very rarely found As a rule children were free from fever though parasites were present in marked numbers Occasionally they suffered from slight attacks of fever When fever was present it was of quotidian type though the parasite found was malignant tertian

Sometimes there was an alternation between the fever of quotidian type and of *tertian type as in the case No 9* named Aphoo, a female aged 4 years described in my paper This was evidently due to the rise and fall of the resistance to malaria as elucidated by Colonel James The duration of quotidian fever was much prolonged and often disappeared without development into the tertian form as described by Colonel James

The fever in hill children when present was always of the intermittent type as a rule quotidian and in a few cases tertian This is described in the following quotation from my paper

The remittent or continuous type of fever which is frequent in the malarial districts of Bengal is not usual among the hill children In fact, during my stay in the district, cases of continued fever noticed by me have occurred, as a rule amongst the non hill tribe population The very few cases of continued fever I have seen amongst the hill children have all occurred in very young children under *two years of age and some of these have been fatal* '

Thus the primary malaria described by *Colonel James* has points of resemblance with the malarial fever prevalent amongst the children of hill tribes in the Chittagong Hill Tracts Now, what accounts for the changing febrile picture described by Colonel James, viz , at first an irregular, then a quotidian lastly a tertian fever ? Colonel James has given the reason for the same

If films taken at four hourly intervals be studied, we find that some parasites lag in their development The blood picture at first one in which every parasite was at the same stage of development becomes confused, with parasites present in all stages of schizogony This finding is always present during the first initial stage with irregular fever

As the fever progresses, two dominant strains of parasites become evident completing their schizogony cycle on alternate days This is associated with quotidian fever and rigors

Lastly, as the terminal stage is reached, the patient's powers of resistance overcome these parasitic broods The brood which is least numerous or less resistant will be overcome first so that the fever will change to a tertian type, due to the surviving brood Finally, even this brood is exterminated or held in check and fever gradually disappears altogether

In the fever of relapse or in benign tertian malaria induced in a patient for a second or subsequent time by the bites of mosquitoes matters are entirely different

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schizogony in blood films from such patients The temperature chart however reflects the schizogony cycle of the parasite strain which is most dominant and the patient's powers *of resistance are apparently* able to suppress effect due to other strains '

In the above explanation of Colonel James it has been supposed that in every attack of malarial fever, whether primary or relapse a kind of biological resistance of the patient having power to suppress to a certain extent the effects of the parasite comes into operation Now the hill tribes living a more strenuous life are likely to develop this biological resistance to a greater degree than people living a more easy life Moreover, these hill people who have not used cinchona alkaloids for generations have had greater opportunities for exercising this biological resistance than more civilized people As the result of these it is likely that the hill tribes as a race have got the power to bring forth this biological resistance more readily to deal with malaria than people of any other race who use artificial help such as cinchona alkaloids for combating malarial infection

In any event I take this opportunity to bring to the notice of all who are interested in the study of malaria that in the Chittagong Hill Tracts we have certain peculiar phenomena relating to malaria the elucidation of which is likely to help us in the proper understanding the question of immunity to malaria

REFERENCES

SARKAR S L (1913) — The Incidence of Malaria in the town of Arambagh *Ind Med Gaz* September

Idem (1916) — Some Studies in Malaria in Nadia District *Ibid* April

Idem (1917) — The Comparative Mortality of the towns in the Nadia District *Ibid* February

Idem (1921) — A Peculiarity in the Spleen Rate as observed in the District of Chittagong Hill Tracts *Ind Jour Med Res* Vol VIII No 4

ROSS SIR RONALD — Report on the Prevention of Malaria in Mauritius

MUIR and RITCHIE — Manual of Bacteriology

CHRISTOPHERS S R (1915) — On the Spleen Rate and other Splenic Indices *Ind Jour Med Res* Vol II No 4

JAMES S P — Report on the First Results of Laboratory Work on Malaria in England

EDITOR *Indian Medical Gazette* (1926) — New Conceptions in Malaria *Ind Med Ga* September

THE EFFECTS OF TREATMENT ON THE INCIDENCE AND DEGREE OF SPLENIC ENLARGEMENT IN AN ADULT POPULATION INFECTED WITH MALARIA

BY

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THERE can be little doubt that the splenic index is a most useful indicator of the amount of malaria in an untreated population. This index, however, loses much of its value when the population examined is subject to anti-malarial treatment. Such exceptions occur in the case of schools, where school quininization is carried out, and in regiments, jails and labour communities where more or less systematic treatment is given or 'prophylactic quinine' is administered.

It has been known for many years that such anti-malarial measures may cause a great reduction in the splenic index, but little or no work seems to have been undertaken to determine how much of the reduction is due to the production of a permanent cure of the disease and how much to treatment causing a mere disappearance of clinical manifestations.

An excellent opportunity arose in the course of the work of the Quinine and Malaria Inquiry of estimating the effects of treatment on splenic enlargement in an adult population. The spleens and the bloods of all the patients were examined weekly over long periods. The patients received treatment when parasites were detected and the examinations were carried out for at least eight weeks after the cessation of all treatment. During the period of observation the conditions were such that the chance of re-infection could be excluded.

Benign Tertian Malaria.

A large number of patients with histories of chronic infections with this disease were observed. These observations were made irrespective of the presence or absence of malarial manifestations or whether malarial treatment was being taken at the time or not. A splenic index of 11.25 was found amongst 1,128 British patients so examined. Subsequent blood examinations proved that at least 46.5 per cent of these people must have been infected with *P. vivax* at the time the index was taken, i.e., four times the number shown by the splenic index.

At the time when the bloods of 1 241 British patients were found to harbour *P vivax* only about 48 per cent showed splenic enlargement The splenic index in these cases fell to seven and the parasite index was nil after a month of daily treatment with one or other of the cinchona alkaloids Subsequent blood examinations showed that in spite of the low character of the indices, yet 60 to 70 per cent of the patients were still infected with *P vivax*

Malignant Tertian Malaria

Of 591 Indian patients, mostly suffering from fresh infections with *P falciparum* the splenic index was 31 6 at the time when parasites were first detected This index fell to 24 8 after one week of treatment with quinine or cinchona febrifuge Of 78 British patients with chronic infections the splenic index was 70 before quinine treatment and had fallen to 24 3 after one week of treatment In both these groups the number of patients who were proved to be still infected after the end of treatment was about 40 per cent

Conclusions —(1) The splenic index in an adult population which receives more or less systematic anti malarial treatment is no true indication of the amount of uncured malaria in that population

(2) The splenic index in such a population probably affords a relative indication of the effect of treatment in controlling clinical manifestations but not in producing a permanent cure of the disease

The Rate of Reduction of Splenic Enlargement following Treatment

Two types of splenic enlargement must be distinguished—the acute and the chronic The large soft spleen found with the acute disease is probably due to an acute congestion of the organ and a lowered vascular tone Such spleens rarely enlarge more than three finger breadths beyond the costal margin and diminish in size very rapidly under the influence of treatment The other type of spleen is the hard form—the typical ' ague cake of chronic malarial infections This form is usually the result of multiple untreated or imperfectly treated attacks or infections Such spleens may attain a great size and they take much longer to return to normal even when the infection is cured This is probably due to a certain amount of increased fibrous tissue produced by the irritation of large amounts of malarial pigment which has been deposited in the stroma of the organ The spleens with old enlargement are also liable to superimposed acute enlargement due to an acute attack of the disease supervening on the chronic infection The additional enlargement due to such attacks usually decreases rapidly when appropriate specific treatment is given

An argument raised against the value of the splenic index is that the figures may include a number of persons whose infections are cured but who still have splenic enlargement An attempt has, therefore been made to determine the rate at which the size of the enlarged spleen returns to normal after a cure has been effected The splenic index was observed weekly in 282 patients who had been

treated for malignant tertian malaria and whom subsequent blood examinations proved to be cured of their infections The splenic index was 40 2 before treat ment and had fallen to 23 4 at the termination of all treatment which lasted only one week A week after the cessation of treatment the index was 12 9, at the fourth week 7 4, at the sixth week about 6 and at the end of the eighth week about 4 3, i e , one tenth of the original index

The size of the average spleen was calculated in this cured population giving a value of one to palpable spleens, two to spleens enlarged one finger breadth beyond the costal margin and so on The average spleen was found to be 1 22 before treatment, 0 76 at the end of treatment and 0 44 after one week of observation From this time it fell gradually to 0 12 at the end of eight weeks of observation, i e , one tenth of the original figure

Both these records show a very sudden drop during treatment and the first week of observation afterwards This is probably due to the rapid reduction in size of the acute enlargement of some of the spleens while the more gradual fall seen later is due to the slower reduction of the chronic enlargements

A number of spleens showing five to seven finger breadths of enlargement were included in this series The sudden primary reduction in size was also noted amongst these and most of them had gradually returned to normal or only one or two finger breadths of enlargement at the end of eight weeks The most rapid reduction in size was observed after the quinine and alkali treatment

Conclusions —(1) These figures go to show, when a malignant tertian infection is cured and re infection prevented, that splenic enlargement, even when considerable tends to disappear comparatively rapidly

(2) One may deduct from these results that persons in an untreated population, who are cured of their infection but still have splenic enlargement, will rapidly lose such enlargement in the absence of re infection

(3) The number of cured cases with splenic enlargement in any unselected population would probably be too small at any one examination to affect the splenic index materially

MEASUREMENT OF THE ENLARGED SPLEEN IN ADULTS

BY

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IN the course of a malaria investigation in the Andamans in 1926 the method for the measurement of the position of the enlarged spleen introduced by Christophers (1924a) was employed in the case of 895 adults and 940 children

This method consists in the triangulation of the apex or most projecting part of the spleen by measurements in centimetres from the umbilicus and from the median line of the body In addition the degree of projection of the spleen below the costal margin is measured so that in any given instance the position of the costal margin is also known The position of the apex of the spleen in relation to the umbilicus in each of a large series of cases may be entered on an abdominal chart the mean position of the series being indicated by some symbol such as a cross At the foot of the chart may be entered the particulars relating to the parasite rate of the community under investigation In this way a chart on which all the essential details with regard to the spleen and parasite rates are shown graphically may be prepared for each section of a town or tract of country

This method has many advantages over the old method of estimating the enlargement of the spleen by means of measurements in finger breadths from the costal margin The umbilicus though not an absolutely fixed point does not vary in position nearly as much as does the costal margin The exact position of the apex of the spleen with regard to the umbilicus is fixed by this system of measurements whilst the preparation of the charts gives all the required information in graphic form which is of the greatest value when comparing the degree of malaria in different communities

As Christophers has pointed out the great point in favour of this method is that it is the most nearly perfect of those hitherto employed The procedure is extremely simple and eminently suited for field work the slight extra expenditure of time involved being amply compensated for by its incomparably greater scientific accuracy

In the case of children the measurements were made in the erect position for in the great majority of instances it is extremely easy to determine the position of

the apex of the enlarged spleen With adults however, it was frequently found difficult to determine the exact position of the apex in the erect position owing chiefly to the greater muscular development of the abdominal wall The men were therefore examined lying down on a perfectly flat surface, such as a form or plank, or the floor of a barrack The abdomen was first palpated with the knees drawn up and then if the spleen were found to be palpable, the measurements were made with the lower limbs completely extended and the arms by the side of the body, in order to secure uniform results At the time of measurement, the man was directed to breathe as quietly as possible, and the measurements were in each case taken at the end of expiration It was found in practice that this procedure actually involved the expenditure of less time than if the men were examined standing owing to the greater ease with which otherwise ' doubtful ' spleens are felt with the subject in the recumbent position

In the study of malarial conditions in any community the accurate enumeration of parasites in the blood taken in conjunction with exact methods of spleen measure ment is of the greatest value, as has been shown by Christophers (1924*b*, 1925) in his researches on the mechanism of immunity in that disease The method of counting the parasites used in the present investigation was with slight modification, that devised by Sinton (1924) 0 055 c mm of blood being examined in each case In this method a small quantity of blood from the finger is drawn up to a mark on a pipette and mixed with an equal quantity of a standard suspension of fowl's blood cells The mixture is then blown out on to a slide and made into three thick drops, and the parasites are counted against the number of fowl's corpuscles observed A fresh pipette must be used for each case, and it was found convenient to use the form known as capillary lymph tubes ' These may be obtained in small boxes each containing 1 000 tubes which may conveniently be carried in the pocket In order to fit a teat to one of these a small rubber cork is inserted into the mouth of the teat, a tapering hole being made through the centre of the cork, into which the end of the pipette is fitted

This method appears to me to be the best yet devised for practical use in the field The technique is simple there is no bulky apparatus to be carried and by its means a definitely known quantity of blood may be searched in each case The actual enumeration of the parasites of course takes time, but, as in the case of spleen measurements a comparatively small number of mathematically accurate results is of infinitely more value than a large number evolved by less accurate methods

Results of Observations on the Enlarged Spleen in Adults

The adults examined have been classed under the following headings —

A Persons with chronic enlargement of the spleen

(i) Those living under hyper endemic conditions in an area where the spleen rate was 65 per cent the parasite rate 20 per cent and the average parasite value 278 per c mm of blood

(ii) Those living under conditions of moderate endemicity in an area where the spleen rate was 17 per cent the parasite rate 4 per cent and the average parasite value 18 per c mm of blood

B Persons suffering from acute enlargement of the spleen the result of recently acquired malaria among whom the spleen rate was 66 per cent the parasite rate 86 per cent, and the average parasite value 3 701 per c mm of blood

The exact figures with regard to the spleen measurements and enumeration of parasites during this investigation were given in detail in a paper by Covell and Baily (1927) The conclusions arrived at from a study of this series of cases were as follows —

1 The normal path of enlargement of the spleen in adults does not appear to differ materially from that observed among children

2 The position of the apex of the spleen in the case of 84 per cent of the individuals examined lay within a distance of 2 cms of a line drawn from the umbilicus to a point 12 cms distant from it and 10 cms to the left of the median line of the body The mean position of the apex in each of the three categories mentioned above lay approximately on this line

3 In the case of chronic infections an increased spleen rate among adults was associated with a greater size of the average enlarged spleen

4 The percentage of parasite infections in adults (and also in children) increased with greater enlargement of the spleen

5 The average parasite value increased with the size of the spleen up to a certain degree of enlargement The size of spleen in adults associated with the highest parasite value was one with the apex situated at a distance of about 6 8 cms from the umbilicus, corresponding with an average costal projection of about 6 cms, which would under the old system of measurement represent a 'three finger' or 'four finger' spleen Spleens of a greater size than this were associated with a progressively decreasing parasite value

REFERENCES

Christophers S R (1924a) The Shape and Position of the palpable portion of the Enlarged Spleen in Children *Ind Jour Med Res* Vol XI 4 pp 1081—1098

Idem (1924b) The Mechanism of Immunity against Malaria in Communities living under Hyper endemic Conditions *Ibid* Vol XII 2 pp 273—294

Idem (1925) Two Malarial Surveys connected with Industrial Projects in certain very highly Malarial Localities *Ibid* Vol XIII 2 pp 343—405

Sinton J A (1924) Methods for the Enumeration of Parasites and Leucocytes in the Blood of Malarial Patients *Ibid* Vol XII 2 pp 341—346

Covell G and Baily, J D (1927) Observations on Malaria in the Andamans, with special reference to the Enlarged Spleen in Adults *Ibid*, Vol XV, 2 pp 309—326

DISCUSSION

Dr J W Scharff (Straits Settlements) I should like to ask Col Christophers how he proposes to measure spleens due to infection with malaria that are enlarged only to an extent which makes them palpable on deep respiration—the so called P I spleens I have recently examined with Dr Russell more than 500 school children in Penang We were in almost complete agreement with regard to spleens enlarged below the costal margin, but we found a difference of between 10 15 per cent in our figures concerning P I enlargement The final result of our enquiry was to more than double the spleen rate that was formerly reached in these schools I understand that the measurement of P I spleens is found to be of great value in determining the true malarial incidence in the Southern States of the U S A , but I wonder if this is the case at present in highly malarial places in the tropics where the more readily comparable results of spleen measurement below the costal margin give us all the data that we require for field work

Dr K E Surbek (Sumatra) I would like to ask Col Christophers if anything is known of the correlation which may exist between the kind of malaria and the incidence of spleen enlargement It has, on another occasion been pointed out by Prof Schuffner that in tropical malaria spleen enlargement is much less frequent than in tertian malaria The same is my personal experience

I would like to ask Major Sinton whether the good effects of iodine treatment in certain forms of splenomegaly is known to him 3×5 to 3×20 drops of Tinct Iodi , pro die, given per os

Major G G Jolly (Burma) Col Christophers has stressed the importance of very careful and accurate spleen measurements , much more accurate than the busy health officer can find time to carry out Major Sinton has pointed out an error that may upset comparisons of spleen rates namely, the question of 'treatment' There is another factor which may vitiate such comparisons , that is the seasonal factor In Ryanh Pyu Burma I noticed some years ago, that a spleen rate taken in 1923 was considerably greater than one taken by Lala in 1912 although the general opinion was that malaria had diminished as a result of anti malarial work and although our statistics showed an improvement In looking for an explanation I noticed that, while the 1912 rate was taken in February I had taken mine in August which is a malarious month A further spleen rate taken in February gave a figure lower than that obtained by Lala in 1912 and much lower than that obtained by me in August 1923 The spleen rate evidently fluctuated according to the season

To elucidate the point further I arranged for a malaria survey to be carried out this year and asked Dr Feegrade, who did it, to watch the spleens of a number of bazaar children under 10 over the period of the survey He did so and the figures obtained are sufficiently interesting to justify putting them before you Out of 56 children whose spleens were examined once a month for the months of July, August September and October 29 spleens diminished steadily in size , 9 decreased and enlarged again 10 enlarged and then decreased , 3 remained the same , 4 steadily enlarged 2 decreased, enlarged and decreased again, while 6 that were enlarged dwindled to normal size

It would appear, therefore that the spleens of children infected with malaria are in a state of flux and that the spleen rate of any particular malarious area varies from month to month and from season to season

If this be so, spleen rates of different places, or of the same place in different years must be regarded as evidence of conditions obtaining at a particular date and cannot correctly be compared unless taken on the same date or preferably at the same stage of the malaria season

Lieut-Col S P. James I M S (Rtd) (Great Britain) I suppose that Col Christophers' method of inquiry and the results obtained are applicable only in hyper endemic malarious areas where the population is entirely untreated with quinine Elsewhere many variable factors would lessen its value The splenic index by whatever method it may be taken, is misleading in countries where malaria has a low endemicity and short seasonal prevalence It is also misleading in areas where quinine treatment is practised and where primary attacks are the chief manifestation of the disease In those circumstances, there may be a considerable amount of clinical malaria but an inappreciable or very low spleen rate which varies at different times of the year Consideration must also be given in those lightly affected places to other diseases and conditions causing temporary enlargement of the spleen particularly syphilis, tuberculosis, rickets, measles, enlarged tonsils and even (as has been found in England) preventive vaccination for smallpox In the Southern States of North America the use of spleen rates as a measure of malaria has not met with general approval because, when this method is carried out in the manner in general use in the Orient it is rare to find a community with an appreciable spleen rate The late Dr Darling, working in the Southern State of Georgia, introduced his method of detecting small degrees of splenic enlargement in an endeavour to overcome that difficulty but its use has not become habitual even in the United States In 1920 it was tried on a rather extensive scale in some malarious localities of Holland but was abandoned because, good as the method is, the number of enlarged spleens found was very small

Dr W C Sweet (Rockefeller Foundation, Mysore) In connection with spleen rates in malaria surveys, certain unpublished results of a spleen survey of the Mysore State, recently completed, may be of interest Due to lack of equipment and trained staff as well as presence of other survey work it was found impossible to proceed with a full malaria survey, so an attempt was made to map the relative malarial endemicity of the various parts of the state by means of a spleen survey

The relative size of spleens found enlarged were recorded by a method recommended by Darling and used in Italy in Hackett's work Darling's classification had an initial class of slightly enlarged spleens which did not descend quite to the costal margin on deep inspiration but were palpable by careful examination This class was omitted as it was not thought to be significant in countries like India where high malarial endemicity is frequently found Our classification began there with a class 'P' in which were spleens which descended to the costal margin on deep inspiration The area from the costal margin to the umbilicus was divided into three portions and spleens were classified as 1, 2 or 3 according to the examiner's judgment of their relative positions Spleens between the umbilicus and the anterior superior iliac spine were also placed in three classes, 4, 5 and 6, the ones placed in class 6 being the largest possible The

examinations were made with the patients lying down with knees flexed and were all made by one person The children's ages ranged from 2 to 15 years

This classification bears some relation to the size of the child and is, therefore, thought to be preferable to the more usual finger breadth system That is instead of applying an arbitrary standard, finger breadths, to all children regardless of size we have a simple method of correlating the size of the spleen to an oblique measurement of the abdomen which Col Christophers has shown to have a quite definite relation to the size of the child Not having used Col Christophers' more detailed measurements, I am not in a position to compare the two methods

The spleen survey of Mysore made it possible to map out the State, quite accurately, into four areas In the first (one district only) there is no endemic malaria but histories indicate the rare occurrence of mild epidemics The second zone is made up of two districts in which endemic areas are rare, a further two districts give a more or less uniform endemicity with spleen rates up to 50 per cent, the fourth zone of three smaller districts in more hilly country has a uniform high endemicity in most places with over 80 per cent spleens During the survey upwards of 8,000 children were examined and about 1,100 miles travelled

As a result of this survey we now have some idea of the distribution and magnitude of our malaria problem and can proceed to a more intensive study of intelligently selected areas in which demonstration control centres may be attempted

Each child examined was questioned as to a previous history of having had chills no mention being made of fever The 'chill rates' obtained give a correlation with spleen rates found of about 0 8 plus or minus an insignificant probable error

Prof J W W Stephens (Great Britain) Col Christophers must be congratulated on a lucid account of a complex question While having nothing to contribute bearing directly on the subject I would venture to point out that the nature of spleen enlargement is obscure and this is necessarily so, I think because the structure of the normal spleen is still very imperfectly known Is enlargement due to engorgement, and if so of what—spleen pulp or splenic sinuses or of both? If it is due to hyperplasia, of what cells—reticulum cells of the pulp or endothelial cells of the sinuses or of both? Another factor of importance is that the spleen varies in size It is estimated that after exercise it can disgorge up to 200 c cs of blood

(Major Sinton's paper) In clinical practice in cases returning from India with a history of malaria we encounter enlarged spleens (say to the umbilicus) They are not due to kala azar or Banti s disease or any other condition that can be diagnosed Are they old malaria spleens that have not 'gone down'?

Bt-Col. S R Christophers, I M S, (B India) replied The type of spleen referred to by Dr Scharff has not so far come into special prominence in India where the definitely enlarged organ has mostly been dealt with No difficulty arises in studying this class, however, by the method of frequency distribution I have described Such spleens for the present, however, though to be recorded are probably best omitted if a single mean figure is required to express the average spleen Such spleens may represent the effects of short transient or cured infections as distinct from the type of prolonged infection seen in the more highly endemic areas and so may have a special importance under particular conditions

The relation of degree of enlargement of the spleen to the different forms of parasite raised by Dr Surbek has often attracted my attention but the question is difficult to decide owing to the almost normal state of double or triple infections in children in endemic areas in the tropics On the whole I suspect as does Prof Schuffner that the enlargement is greatest with simple tertian or quartan especially the latter but it is difficult to substantiate this

With regard to what Major Jolly has said it is rather against my own experience that great change takes place in individual spleens in children in highly endemic areas over short periods of time My original expectation was that such changes could be a feature of the enlarged spleen but actual observation of individual children carried out at intervals for a period of 9 months in the highly malarious village of Singanama in the Central Provinces showed to my surprise extraordinarily little change in the individual One had, therefore to modify one's view of a ceaseless kaleidoscopic change in the spleens of a community as necessarily present Possibly the conditions in this respect are different with more moderate spleen rates or even with high spleen rates under different circumstances Seasonal variation in the spleen in communities is a proper study for the scientific malariologist All such issues require working out before full advantage can be taken of the spleen rate for practical purposes It is my experience that any such study is almost valueless without a satisfactory system of measurement which need not necessarily be the one finally used in practice

It is interesting to have heard Dr Sweet's experiences of the value of the spleen rate in mapping endemic malaria over large tracts The method of measuring the spleen by proportionate relation to equal sub divisions of a costal margin umbilicus line as indicated by Dr Sweet (and in a somewhat different form as employed by Prof Schuffner) has theoretically the great advantage of simplicity One objection to be borne in mind is that since the majority of apices will lie as I have shown at a distance between 6 and 10 cms from the umbilicus they will preponderatingly fall within Dr Sweet's No 2 portion of the costal margin umbilicus line and so delicacy of measurement is lost just where it is most required, i e, like so many other systems of measurement this is apt just to miss that very small actual variation which is present and tell us only what we could now with practical certainty predict namely that the average apex under any circumstances seen in endemic malaria will lie somewhere about a particular point Prof Schuffner's midway division of the costo umbilicus line crosses almost over the middle of the modal area and consequently slight individual bias of the observer may easily throw a large proportion of the spleens on this line to one or other side of the line as the case may be The measurement in centimetres is really a very simple matter though it sounds complicated and is practised as a routine now by most of our malaria department investigators Its results are devoid of the ambiguity of the so called simpler methods

MALARIA : TREATMENT.

EXPERIMENTS ON THE TREATMENT OF MALARIA IN ENGLAND

BY

LIEUT COL S P JAMES, M D , I M S (RETD)
British Ministry of Health London,

W D NICOL

AND

P G SHUTE

I WISH in the first place to refer to some studies which are different in some respects from those that are usual in connection with research on the treatment of malaria They are concerned not so much with the action of quinine or other drugs on the malaria parasite, as they are with the natural processes or artificial conditions which protect certain individuals from the usual clinical and parasitological effects of a malarial infection, or which free some individuals very quickly from those effects without the assistance of quinine or other drug It seems possible that, if more knowledge of those natural processes or artificial conditions were available, it might lead to practical measures of material assistance in limiting the present importance of malaria as a cause of sickness and death

One of the most striking results which has emerged from work on induced malaria in England is that a malarial attack does not always result when a person is bitten by Anopheles which are proved to be infective by finding sporozoites in their salivary glands after they have bitten the patient At first there was a difference of opinion on this point Yorke and Macfie reported that, in their experience of 41 cases, the bites of a mosquito which, immediately after the meal, was proved by dissection to contain sporozoites in the salivary glands, had never failed to result in a malarial attack within the usual incubation period of the disease In our observations, however, there had been, up to April 1926, 52 failures to develop malaria among 221 patients bitten by mosquitoes of infective batches, and our total figures up to the middle of September 1927 are 169 failures among 576 patients For some time we tried to explain our failures on technical rather than on

biological grounds but later we were able to prove conclusively that it is quite true that not everyone who receives a dose of sporozoites develops an appreciable malarial attack within the usual incubation period of the disease The proof came when some of our patients who had been reported as having 'failed to take,' and in whom a re inoculation was not done developed a true malarial attack some months after they were bitten Here are temperature charts of two of these cases

It is seen at once on these charts (Charts I and I*a*) that the individuals did not show the effects of the infection during the period when a primary attack was due but that nine and six months later they developed a typical attack In this case no sign or symptom indicated that the inoculation by mosquito bites had successfully infected the patients

Equally interesting are a number of cases with a modified or abortive primary attack from which the patients recover without the attack being observed clinically or if it is observed clinically, without parasites being found in the blood and without any quinine treatment (Charts II and III) In these cases as in the case first mentioned the occurrence of an obvious malarial attack some months later proved that the patient had been successfully infected when he was originally bitten

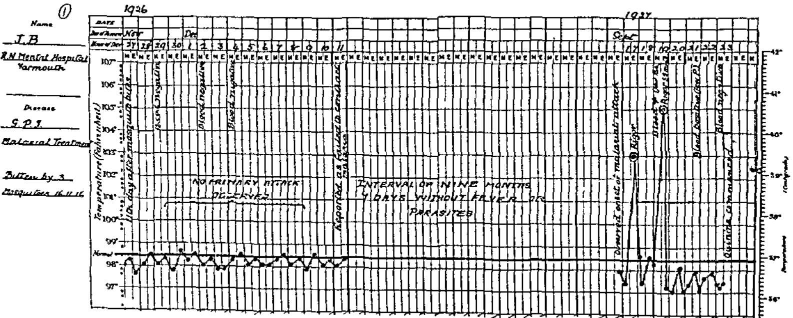
Of a similar type are cases in which the primary attack is observed clinically and by finding parasites in the blood but in which there is so called spontaneous recovery in a few days without quinine treatment (Chart IV)

These charts of course relate to persons who had never previously suffered from malaria They had never been out of England It has to be admitted that at the time of their inoculation some natural process or artificial condition was at work which prevented the development of the malarial infection

Quite a different subject is the condition of immunity to a strain of the benign tertian parasite which, it must now be admitted occurs in individuals who as a therapeutic measure are given two or three courses of malaria induced either by mosquito bites or by direct blood inoculation The usual events in these cases are that the attack caused by the second inoculation dies out spontaneously after a few febrile paroxysms and that the attack caused by a third inoculation either fails entirely or only shows itself by the presence of a few parasites without fever or other clinical manifestation (Chart V)

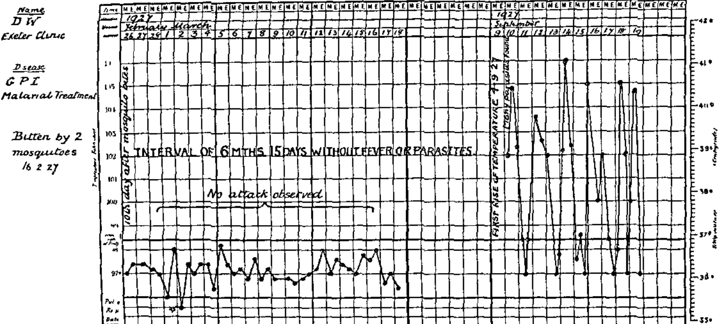
This subject is obviously very important not only in connection with experiments on the treatment of malaria but also from the epidemiological point of view At Horton we have made the surprising observation that patients who have been rendered so immune to our strain of *P vivax* that they can be repeatedly bitten by many infected mosquitoes without showing any clinical or parasitological evidence of infection can be readily given another attack of benign tertian malaria with the usual incubation period and the usual clinical and parasite findings if they are inoculated with a *different strain* of the same species of parasite (*P vivax*) As a rule however, the attack due to this different strain dies out spontaneously after a short series of febrile paroxysms Chart V*a* is an interesting example

CHART I



Name
J.B
R.N Mental Hospital
Yarmouth
Disease
G.P.I.
Malarial Treatment
Bitten by 3
Mosquitoes 16.11.26
1926
Nov
Dec
27
28
29
30
1
2
3
4
5
6
7
8
9
10
11
1927
Sept
17
18
19
20
21
22
23
Normal
107°
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
Temperature (Fahrenheit)
42°
41°
40°
39°
38°
37°
36°
Centigrade
11th day after mosquito bites
Blood negative
Blood negative
Blood negative
NO PRIMARY ATTACK
OBSERVED
Reported as failed to contract malaria
INTERVAL OF NINE MONTHS
4 DAYS WITHOUT FEVER OR
PARASITES
Observed onset of malarial attack
Rigor
Rigor
Blood positive (B.T.P.)
Blood negative
Quinine commenced

CHART Ia



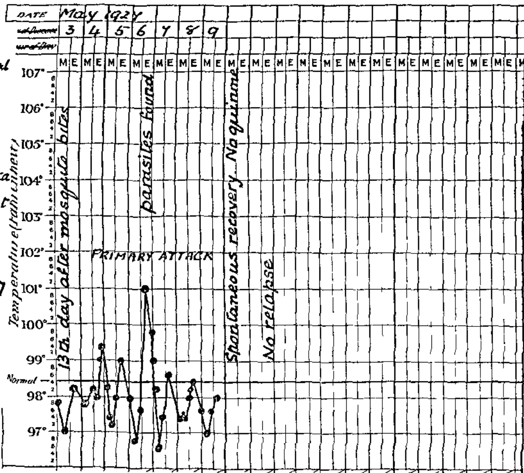
Name
D W
Exeter Clinic
Dsease
G P I
Malarial Treatment
Bitten by 2
mosquitoes
16 2 27
1927
February March
26 27 28 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
1927
September
9 10 11 12 13 14 15 16 17 18 19
10th day after mosquito bites
INTERVAL OF 6 MTHS 15 DAYS WITHOUT FEVER OR PARASITES.
No attack observed
FIRST RISE OF TEMPERATURE 4-9 27
Many parasites found

Mental Hosp.
Disease
Encephalitis Lethargic
Malarial Treatment
Bitten by 3
Mosquitoes 3.12.26
UNOBSERVED
ABORTIVE PRIMARY
ATTACK
Recorded as Failed to contract malaria
INTERVAL OF SEVEN MONTHS
23 DAYS WITHOUT
FEVER OR PARASITES
OBSERVED ONSET OF RELAPSE
PV PARASITES FOUND
QUININE COMMENCED
X grs
41°
40°
39°
38°
37°
36°

CHART III

Name (3)
A E K
West Park
Mental Hospital
Disease
Encephalitis Lethargica
Malarial Treatment
Bitten by 3
Mosquitoes 3.12.26
DATE Dec 1926
Time of Reading M E
Hour of Day 24 25 26 27 28 29 30 31 1 2 3 4 5
JANUARY 1927
August 1927
1 2 3 4 5 6
107° 106 105 104° 103 102° 101 100° 99° Normal 98°
Temperature (Fahrenheit)
42 41 40° 39 38° 37°
Temperature (Centigrade)
OBSERVED ABORTIVE
PRIMARY ATTACK
No parasites found
Spontaneous recovery. No quinine
INTERVAL OF SIX MONTHS
26 DAYS WITHOUT
FEVER OR PARASITES
OBSERVED ONSET OF RELAPSE
QUININE COMMENCED
grs X t.i.d. (8.27)

CHART IV



Name
H. El.
West Park Mental
Disease.
Encephalitis Lethargica
Malarial Treatment
Bitten by 2
Mosquitoes 20.4.27
DATE
May 1927
3 4 5 6 7 8 9
13th day after mosquito bites
parasites found
PRIMARY ATTACK
Spontaneous recovery. No quinine
No relapse
Temperature (Fahrenheit)
107° 106° 105° 104° 103° 102° 101° 100° 99° 98° 97°
Normal
Temperature (Centigrade)
42° 41° 40° 39° 38° 37° 36°

CHART V

ES

C.P.I.
Malarial
Treatment

Bitten by 3
Mosquitoes
15.6.26

PRIMARY
INFECTION

Blood film negative

Interval of
24 days
without fever
or parasites

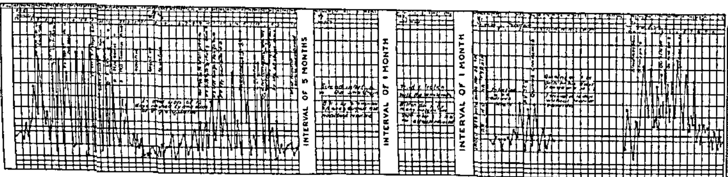
SECOND
INFECTION →
Bitten by 20
mosquitoes

12th day after being bitten

No QUININE

Blood negative

CHART Va



It is evident that each of the observations I have mentioned has an important bearing on the treatment of malaria which we cannot afford to neglect in experimental work—particularly in work on the action of quinine and other drugs It seems from these observations as though a chief aim of experimental work should be to ascertain how to assist the physiological protective and curative processes which many individuals seem naturally to possess In some individuals there is such a nice balance between the natural protective or curative power and the effects of the parasitic invasion that it can be influenced by very slight external stimuli such as warmth and cold, exercise and rest It is curious that nearly all our 'failures to take' have happened during the winter months and that during those months some patients who were kept in bed in a warm room throughout the incubation period developed the disease while others who were allowed to be up and about in the cold failed to do so Cold weather seems to assist the natural curative processes and in this connection one is reminded of the common observation that patients suffering from tropical malaria due to *P falciparum* become free from their infection very quickly in the cold climate of England As regards the effect of exercise, we have a patient who keeps free from fever and parasites while she remains at rest in bed but gets a relapse a day or two after she is allowed up and takes exercise (Chart VI)

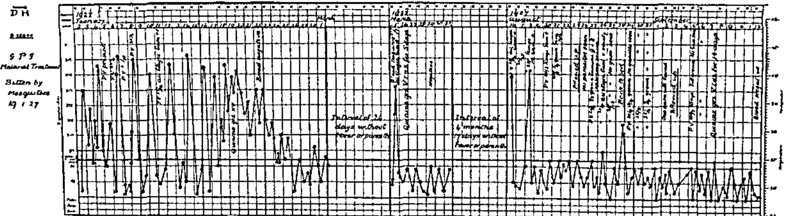
In our experiments on the use of quinine for the treatment of malaria we are trying to follow up the ideas just indicated by endeavouring to ascertain the utility of the drug as a stimulus of the natural curative processes rather than as an agent in killing the malaria parasite

The first chart (Chart VII) shown in this connection illustrates our practice of stopping attacks of therapeutic malaria about the middle of their course by giving the patient one dose of 5 grains of quinine This single dose causes the attacks of fever to cease almost at once and it causes the parasites to disappear from the peripheral blood within two to four days It seems to set in motion some natural process of cure which continues for a considerable time after all the quinine has been eliminated But the cure is not complete for after an interval of freedom from fever and parasites which corresponds rather closely to the incubation period of the primary attack there is a recrudescence resembling the primary attack but usually less severe

Other results which we have ascertained regarding the effect of a single dose are —(1) A single dose even of 30 grains has no effect if given at any time during the incubation period of the disease even on the day before the first rise of fever (2) The single dose, given about the middle of the attack must be sufficiently large but by increasing it beyond that amount no better effect is obtained For example, a dose of 2 5 grains has usually no curative effect and a dose of 10 grains or a dose of 20 grains has usually no greater curative effect than a dose of 5 grains

On this point the next chart (Chart VIII) shows how ineffective it is to give quinine during the incubation period or even on the first day on which fever appears

CHART VI



D H
G P I
Malarial Treatment
Bitten by
Mosquitoes
19 1 27
February
March
August
September
Blood negative
Interval of 24 days without fever or parasites
Interval of 4 months 17 days without fever or parasites

The next charts are examples of recrudescences and relapses If there is a return of fever and parasites within six weeks of an attack we call it a recrudescence if the interval is longer than six weeks we call it a relapse I may say at once that recrudescences after a relapse are more frequent than recrudescences after the primary attack

CHART IX

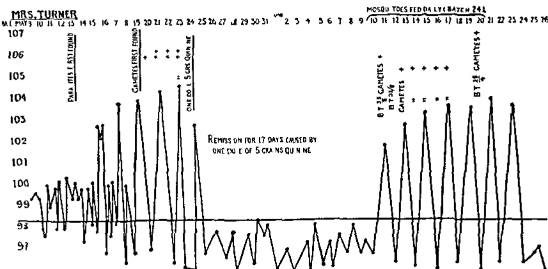


Chart IX illustrates one recrudescence after the primary attack and three recrudescences after the true relapse

The treatment in this case was 10 grains three times a day for ten days in the primary attack and 10 grains three times a day for five days in the true relapse No quinine was given for the treatment of any of the three recrudescences following the true relapse recovery from them occurred without any treatment and the patient has not since suffered from any symptom of a recurrence of the disease

The next chart (Chart X) is of a case in which the interval between the primary attack and the recrudescence was as long as five weeks (34 days)

Both the primary attack and the recrudescence were treated with 10 grains of quinine three times a day for five days The patient had a true relapse six *months after the recrudescence but she recovered from it without quinine and* has not since had a recurrence

So much for recrudescences Now I would like to show a few charts illustrating true relapses These relapses are more interesting than recrudescences because it seems probable that their causation is entirely different A reasonable explanation of a recrudescence is that not all the parasites in the red blood corpuscles

CHART VIII

Name A R

Horton Mental Hospital

G P I

Malarial Treatment

1927 August 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 September 1 2 3 4 5

On all these days the patient was given 5 gr. quinine twice (morning and evening)

Blood film negative

CHART IX

M M
Horton Mental Hospital

G P I
Malarial Treatment

Bitten by 40 Mosquitoes
27 11 25
Bitten by 12 Mosquitoes
1 12 25

Primary Attack

Interval of 28 days without fever or Parasites

Interval of 6 mths 18 days without fever or parasites

Interval of 25 days without fever or parasites

Interval of 28 days without fever or parasites

Interval of 18 days without fever or parasites

No Quinine

CHART X

E D
Horton Mental Hospital

G P I
Malarial Treatment

Bitten by 50 Mosquitoes
14 10 25

Interval of 5 months without fever or relapse

Observed onset of relapse

Interval of 6 months without fever or parasites

Observed onset of relapse

Blood negative

No quinine

have been killed or have died a natural death but this explanation is not reasonable for a relapse which suddenly occurs without any warning eight months after a primary attack which may have been so mild as to be unobserved It is very curious that such a high proportion of these true relapses occur at about the eighth or ninth month after the primary attack This fact recalls to mind various articles in the early literature of experimental malarial infections particularly an article by P Thurburn Manson entitled Experimental Malaria recurrence after nine months published in the *British Medical Journal* of 13th July 1901, and an article by Major C F Fearnside I M S entitled 'Experimental inoculation of malaria with a relapse after eight months' published in the *Indian Medical Gazette* of January 1903 From the point of view of the present paper the chief interest of these cases was that the primary attack was treated with large daily doses of quinine and that after recovery from the attack quinine treatment was continued for a long period—apparently at least three months

In the first chart of our cases (Chart XI) shown the primary attack was treated with 10 grains of quinine three times a day for five days No further quinine was given The second chart (Chart XII) is of a patient whose primary attack was treated with 5 grains of quinine three times a day for ten days For the last two charts shown (Charts XIII and XIV) cases have been selected in which the relapse occurred at an interval of about six months They are noteworthy also on account of the large doses and long duration of quinine treatment in the primary attack the relapse and in the second case the recrudescences which follow the relapse

Comparing these charts with those previously shown it is evident that a plan which would usually be termed thorough and prolonged quinine treatment and after treatment of the primary attack has no more effect in preventing a true relapse than has a plan which until recently would have been condemned as being quite inadequate

In conclusion I should like to make it clear that in my opinion it would be a great error to assume that the results obtained in England would be equally applicable to the treatment of malaria in tropical countries I feel very strongly that until we know more of the natural processes and artificial conditions governing the so called 'spontaneous cure of malaria and its failure to develop as a clinical disease in some classes of individuals and in some climates or seasons we must regard its treatment by drugs as being a local problem quite as truly as is any other public health method which has been tried or suggested for dealing with the disease We do not know at all whether such small doses of quinine as are effective, let us say in England or Holland or the United States of America would be equally effective among the people of India or of West Africa It seems as though a long series of local researches on this subject would be necessary and I do not see how they can be avoided

CHART VI

Name
M S
HORTON MENTAL HOSPITAL

Disease
G P I
Malarial Treatment

Bitten by 11
Mosquitoes 9 3 26
" 31 3 26

1926 April 12 13 14 15 16 17 18 19 20 21 22 23 24 25

INTERVAL OF 8 MONTHS
2 DAYS WITHOUT FEVER
OR PARASITES

1926 December 27 28 29 30 31 — 1927 January 1 2 3 4 5 6 7 8



ave been killed or have died a natural death but this explanation is not reasonable for a relapse which suddenly occurs without any warning eight months after a primary attack which may have been so mild as to be unobserved It is very curious that such a high proportion of these true relapses occur at about the eighth or ninth month after the primary attack This fact recalls to mind various articles in the early literature of experimental malarial infections particularly an article by P Thurlurn Manson entitled Experimental Malaria recurrence after nine months published in the *British Medical Journal* of 13th July 1901 and an article by Major C F Fearnside I M S entitled Experimental inoculation of malaria with a relapse after eight months published in the *Indian Medical Gazette* of January 1902 From the point of view of the present paper the chief interest of those cases was that the primary attack was treated with large daily doses of quinine and that after recovery from the attack quinine treatment was continued for a long period—apparently at least three months.

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CHART VI

Name
M S
HORTON MENTAL HOSPITAL

Disease
G P I
Malarial Treatment

Bitten by 11 Mosquitoes 9 3 26 + 31 3 26

1926 April 12 13 14 15 16 17 18 19 20 21 22 23 24 25

INTERVAL OF 8 MONTHS 2 DAYS WITHOUT FEVER OR PARASITES

1926 December 27 28 29 30 31 — 1927 January 1 2 3 4 5 6 7 8

Negative



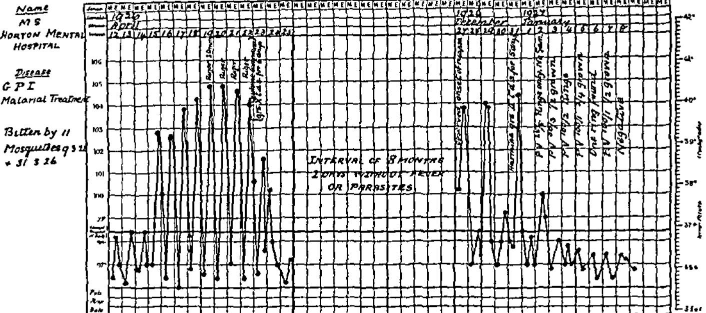
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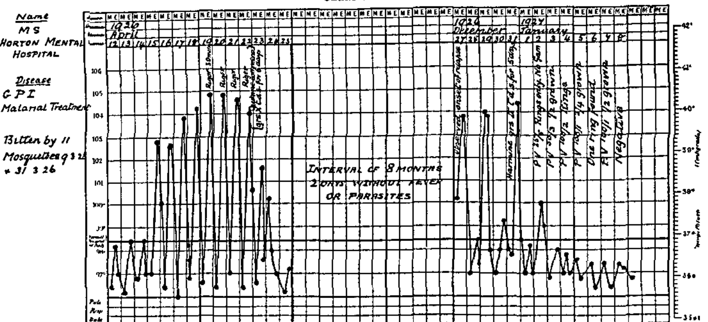
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CHART XI



Name
M S
HORTON MENTAL HOSPITAL
Disease
G P I
Malarial Treatment
Bitten by 11
Mosquitoes 9 3 26
+ 31 3 26
1926
April
1926
December
1927
January
INTERVAL OF 8 MONTHS 2 DAYS WITHOUT FEVER OR PARASITES
Negative

Chart XI



Name
M S
Horton Mental Hospital
Disease
G P I
Malarial Treatment
Bitten by 11 Mosquitoes 9 3 26
+ 31 3 26
1926
April
1926
December
1927
January
Rigor
Observed onset of relapse
PV 25/1 Rings only. No Gam
PV 30/3 1/2 grown
PV 100/2 Rings
PV 100/1 3/4 grown
One ring found
PV 100/1 1/2 grown
Negative
INTERVAL OF 8 MONTHS 20 DAYS WITHOUT FEVER OR PARASITES

THE TREATMENT OF MALARIAL FEVERS

BY

MAJOR J. A. SINTON, V.C., O.B.E., I.M.S.,
Central Malaria Organization, Kasauli.

ANY discussion on the treatment of malarial fevers must take into account the fact that 'malaria' is not a single disease but, at least, three different fevers— *malignant tertian malaria, benign tertian malaria and quartan malaria.* *The action* of different drugs, with regard to both temporary and permanent cure, has been found to vary very considerably in these three diseases.

POINTS OF AN IDEAL TREATMENT OF MALARIA

The essential points in an ideal treatment of malaria are, in my opinion, the following :—

(1) It should bring about a rapid cessation of the symptoms complained of *by the patient and of any acute condition, which is likely to be dangerous to his life.*

(2) It should cause no harm to the patient.

(3) It should destroy all the parasites in the body or, at least, bring about such a condition that the natural defences of the body can complete the destruction, thus preventing the recurrence of clinical symptoms with re-invasion of parasites into the peripheral blood at a later date.

(4) It should rapidly destroy all the sexual forms of the parasite in the peripheral blood and prevent their reappearance there, i.e., prevent the patients becoming potential sources of mosquito infection.

(5) It should, if possible, be effective against all the different species of malaria parasite.

(6) It should be capable of being used on a large scale, especially amongst an uneducated and uncontrollable population, such as is common in the tropics. For this purpose it should, therefore, be (*a*) cheap in price, (*b*) have little taste or disagreeable effects, and (*c*) be quick in action, for such a population will usually not return for further treatment after the cessation of clinical symptoms.

PRECAUTIONS NEEDED IN TESTING THE EFFICACY OF ANY LINE OF TREATMENT

Great confusion has arisen in the past as to the value of different drugs in producing a permanent cure in malaria, because their effects have not been tested

in a scientific manner in many instances The following measures are suggested as the main precautions to be taken in testing the efficacy of any line of treatment in producing a permanent cure —

(1) That the disease being treated is malaria diagnosed not merely by clinical signs and symptoms but by the finding of parasites immediately before the commencement of treatment

(2) That the patient has no other disease the effects of which might obscure the action of the treatment being tested

(3) That the drug being tested is actually being taken and retained in the amounts prescribed

(4) That no other drug is being taken at the same time the effects of which might vitiate the results of the experiment

(5) That in comparing different treatments infections due to different species of malaria parasite are considered separately

(6) That a sufficient number of patients are treated in order that the results may not be vitiated by errors of chance distribution

(7) That in comparing the effects of one treatment with another the populations treated by the different methods should be as far as possible homogeneous

(8) That controls should be used to eliminate as far as practicable, any possible variations in the results due to personal bias season altered virulence of the parasites chronicity of infection immunity etc

(9) *That a strict standard as to what is to be considered a permanent cure of* the infection is laid down and if this standard depends on a period of observation chances of re infection should be excluded during this time

(10) That the finding of parasites in the blood is the only true criterion of relapse

These precautions have been used during the experiments detailed in this paper Fuller details have been given in another place (Sinton 1926)

The Treatment of Malignant Tertian Malaria

The Production of a Permanent Cure

Amongst patients infected with *P falciparum* we have been unable to detect any differences between the ease with which fresh infections are cured as compared with chronic ones

A Cinchona Alkaloids

Table I gives a summary of the results of the treatment of over 800 patients infected with this disease About 100 of these patients were European and the remainder Indian

TABLE I

Results of the Treatment of 814 Cases of Malignant Tertian Malaria
(Cinchona Alkaloids)

Treatment *	Daily dose of drug	Total amount of drug given	Duration of treatment	Total cases treated	Cases not observed to relapse but lost sight of	Actual number of relapses observed		Percentage of cases which relapsed: Observed †		Percentage of cases which relapsed: Total †		
	Grains	Grains	Days			B T	M T	B T	M T	Possible maximum	Observed minimum	Average.
QA	30	110–210	4–7	298	41	25‡	39	9 7	15 2	35 2	21 5	27 3
Q	30	110–210	4–7	237	32	14	112	6 8	54 6	66 6	53 1	60·4
CFA	30	140	5	119	14	2	41	2 0	39 4	48 0	36 0	41 9
CF	30	140	5	120	14	2	51	2 0	48 1	55 8	44 1	50·0
QA§	30	110–210	4–7	40	0	0	1	0 0	2 5		2 5	2 5
GRAND TOTAL	30			814	101	43	244	6 0	34 2	47 6	35 2	41 1

* QA Is quinine and alkali treatment Q Is quinine only CFA Is cinchona febrifuge and alkali
CF Is cinchona febrifuge only [*vide* Sinton (1926) *Ind Jour Med Res*, Vol XIII, No 3 pp 579 to 601]

† For explanation of these percentages *see* Sinton (1926), p 583

‡ Nineteen of these cases were observed for long periods and showed no further signs of infection with *P falciparum*

§ These patients were observed clinically for 8 weeks and by blood examination at the end of that time as well as whenever clinical symptoms suggested relapse

It can be seen from this table that in our experiments the quinine and alkali treatment described by me (Sinton 1926) gave much better results than any of the other treatments tried It was found as the duration of this treatment rose from 4 to 7 days and with it the amount of quinine given that the percentage of relapses due to *P falciparum* fell from 26 per cent with 4 days of treatment to about 16 per cent with 5 days of treatment and was only about 5 per cent with 7 days treatment

This form of treatment has been tested under varying conditions with both European and Indian patients and has still been found to give uniformly good results with the type of malignant tertian malaria seen in Northern India It is therefore recommended as the treatment of choice in this disease if the conditions are suitable for carrying it out satisfactorily

B Plasmochin and Plasmochin Compound

Six patients were treated with plasmochin and five with plasmochin compound over periods of 7 to 14 days and of these nine or 81 per cent relapsed Of six control cases given quinine and alkali for one week none relapsed

These figures are too small to generalize upon but tend to be in agreement with that of several other workers who have found these drugs of little value in the production of a permanent cure in malignant tertian malaria

The Production of a Clinical Cure

In recording the effects of any drug in producing a clinical cure one should exclude as far as possible the presence of any intercurrent disease which will obscure the results of treatment It has been found that malaria in India may be complicated by or may complicate almost every disease known in the tropics When the presence of any such intercurrent disease was detected amongst our patients the patient affected was excluded from the observations

A preliminary purgation with calomel followed by magnesium sulphate was given to all patients before treatment commenced and care was taken to keep the bowels open afterwards This we believe helps to account for the good results obtained

Fever —The average duration of fever after the commencement of treatment with the cinchona alkaloids (*vide* Table I) was about 0 61 days *per case* This result was obtained from the observation of 849 Indian patients suffering from malignant tertian malaria chiefly fresh infections In only 45 or 5 3 per cent did fever last more than 3 days and in none more than 4½ days

The average duration of fever in 81 British patients with chronic infections was 0 23 days *In only one case did the fever last 3½ days* The duration of fever in these chronic cases was less than in the fresh infections

Many of the chronic cases were said to be quinine resistant or to have relapsed while taking 30 grains of quinine daily We took special precautions to ensure that all patients received and retained the amounts of the drug prescribed and

we have never seen a case relapse while taking quinine in such doses nor found a quinine resistant case

The patients treated with alkali showed a slightly shorter duration of fever than those who did not get this adjuvant

Spleen —The spleen rate before the commencement of treatment in 669 patients was 36 per cent and had fallen to 25 per cent after one week of treatment A more marked reduction occurred amongst the patients treated with alkali than in the other patients

The Effects of Treatment on the Sexual Forms of P falciparum

A *Cinchona Alkaloids* —It has long been known that the cinchona alkaloids appear to exert little destructive action on the gametocytes of *P falciparum* Of 618 cases of this fever observed 6 9 per cent showed crescents before the commencement of treatment and 25 2 per cent at the end of a treatment lasting 4 to 7 days with either quinine or cinchona febrifuge in doses of 30 grains daily (Sinton, 1926) It was found that crescent carriers were fewer after the quinine and alkali treatment than after the other treatments mentioned above

B *Plasmochin and Plasmochin Compound* —Five crescent carriers were treated with these two drugs and the crescents were found to disappear in 1 to 5 days in every case These results though few in number, tend to confirm the assertions of other workers that plasmochin has a specific action on crescents

Conclusions —Under the conditions of these experiments it was found (*a*) that treatment by the quinine and alkali method for 1 week produced a permanent cure in about 80 per cent of the type of infection with *P falciparum* seen in Northern India (*b*) that treatment with plasmochin did not give good results in the production of a permanent cure in the few cases of malignant tertian malaria treated, and (*c*) that plasmochin seems to have a marked and specific destructive action on crescents which is not the case with the cinchona alkaloids

It is recommended that in the case of crescent carriers and of heavy infections with *P falciparum* a treatment for 5 days with plasmochin should be given concurrently with or following upon one week of treatment with quinine and alkali

THE TREATMENT OF BENIGN TERTIAN MALARIA

I *The Production of a Permanent Cure in Fresh Infections*

The opinion formed by many workers during the war and afterwards was that fresh infections with *P vivax* were more easily cured than chronic ones The results of the treatment of artificially induced infections for the cure of general paralysis of the insane seem to bear out this belief

Thirty four British patients suffering from fresh infections with *P vivax* were treated with different cinchona alkaloids Of these patients 3 relapsed and 1 was lost sight of giving an average relapse rate of about 10 per cent Of these

patients, 25 were given quinine treatment and only one, or 4 0 per cent, relapsed

A series of Indian patients were treated, amongst whom a comparatively large number were suffering from fresh infections Of 88 of these patients, each of whom received a total of 110 grains of quinine in 4 days about 34 per cent relapsed The result in these cases is higher than those obtained in the previous series or those reported in artificially induced malaria This may be due to the inclusion of chronic cases in the series to the short duration of treatment, or to the fact that during part of the period of observation these patients were exposed to reinfection

These results if compared with those obtained in chronic infections tend to confirm the idea that fresh infections with *P vivax* are more easily cured than chronic ones

II *The Production of a Permanent Cure in Chronic Infections*

More than 1 200 British patients suffering from chronic infections with *P vivax* have been treated during the last 3½ years Under the controlled conditions mentioned previously various methods of treatment have been tested with the results given below

A Cinchona Alkaloids

In Table II are given the results of the treatment of nearly 1 000 patients with different alkaloids

The best average results in our experiments were those given by quinine and cinchonine while the worst were those of quinidine

It was not found that the combination of alkali with any of those alkaloids had a markedly beneficial effect in preventing relapse over that of the drug given alone as long as the bowels were kept well opened with magnesium sulphate

The percentage relapses with the different cinchona alkaloids is very similar to the 60 to 70 per cent of relapses found after quinine treatment by Stephens and his fellow workers at Liverpool during the war in cases of chronic benign tertian malaria

B Stovarsol and its Compounds

(*a*) *Stovarsol* Ten patients were treated with this drug in doses of 1 grm daily by the mouth for 3 days They all relapsed

(*b*) *Stovarsol and Quinine* —Thirty seven patients were given two courses similar to that described above with an interval of 4 days between them At the same time 30 grains of quinine was given daily in solution for 2 weeks The average relapse rate among these patients was 70 6 per cent

TABLE II

Results of the Treatment of 1,039 Cases of Chronic Benign Tertian Malaria

(Cinchona Alkaloids)

Alkaloid	Treatment Daily dose of drug in grains × days administered	Total amount of alkaloid given (Max and Min.) Grains	Total number of cases treated	Cases not observed to relapse but lost sight of	Actual number of relapses observed	Percentage of cases which relapsed			
						Observed	Total		Average.
							Possible maximum	Observed minimum	
Quinine	30 × 14, 10 × 42 30 × 7, 20 × 7	840 350	542	59	334	69·1	72·5	61·5	67·7
Quinidine	20 × 28 20 × 14	560 280	208	14	164	84·5	85·6	78·8	83·0
Cinchonine	20 × 28	560	72	3	47	68·1	69·4	65·3	67·6
Cinchonidine	20 × 28 20 × 21	560 420	107	24	60	72·3	78·5	56·0	68·7
Cinchona febrifuge	30 × 7, 20 × 24 30 × 7, 20 × 21	630 600	110	25	66	77·6	82·7	60·0	73·1
Grand total of Cinchona alkaloids			1,039	125	671	73·4	76·6	64·5	70·2

(*c*) *Quinine Stovarsolate* —Twenty three patients were given this drug in doses corresponding to from 0 53 to 0 64 grm of stovarsol in combination with 0 48 to 0 80 grm of quinine daily for 28 days by the mouth The relapse rate was 70 per cent The larger doses gave a rate of 60 per cent as compared with 70 per cent for the smaller ones

(*d*) *Sodium Stovarsol* —One patient was treated by the intravenous injection of 1 grm of this drug and ten patients with two similar doses separated by a day's interval All these patients relapsed Fourteen patients were given three doses (1, 1½ and 1½ grms) during 5 days and 86 7 per cent relapsed while one patient *given 4 injections (a total of 5½ grms) in 7 days did not relapse* The average relapse rate for these 26 patients was therefore 88 4 per cent

In all ninety six patients suffering from chronic benign tertian malaria were treated with stovarsol and its compounds either alone or in combination with quinine Although various methods of administration were used and the dosage and duration of treatment varied yet an average relapse rate of about 68 per cent has been obtained The discovery of this drug has marked a distinct advance in the treatment of benign tertian malaria and further work with similar compounds may yield valuable results It has not however proved the specific for this disease which it was hoped at one time it might be (Sinton 1926 1927)

C Plasmochin and Plasmochin Compounds

Plasmochin —The treatment originally recommended for this drug was a series of short courses with rests between them The drug was given on 17 days with rests amounting to 22 days a total of 39 days The daily dose of the drug was 0 08 grm plasmochin making a total of 1 36 grms during the treatment Twenty nine patients suffering from chronic infections of *P vivax* were placed on this treatment In two of them treatment had to be stopped on account of persistent toxic symptoms and these with 8 others relapsed at a later date while one case was lost sight of during observation The average relapse rate in this series was 35 3 per cent

It had been suggested that the drug should be administered with as few rests as the toxic manifestations would permit A series of 22 patients were started on a treatment of 0 08 grm daily until 28 days of treatment were completed a total of 2 24 grms per patient Rests were given only when signs of severe toxæmia were observed The time necessary to complete the 28 day course average 35 days (28 to 53 days) Two patients were unable to stand this course and these with three others relapsed after the end of treatment The average relapse rate for these patients was 22 7 per cent

Plasmochin Compound —A series of 15 patients were treated similarly to the first plasmochin series but the dosage of plasmochin was 0 1 grm combined with 1 25 grms quinine daily The total amount of plasmochin given was 2 8 grms and of quinine 35 grms Of these patients treatment had to be stopped in one case and two others relapsed The average relapse rate was 20 per cent

Another series of 20 patients was treated similarly to the second plasmochin series but with a daily dosage of the compound as in the previous experiment. Two patients were lost sight of after the 5th and 6th weeks of observation respectively and none of the others relapsed. The average relapse rate was 3.4 per cent.

Toxic symptoms in the form of cyanosis and abdominal pains were not uncommon in our experience especially when the continuous course of treatment was being tried. One case developed a severe cholera like condition.

Plasmochin was found to have a marked action on *P. vivax* in the peripheral blood. In no case could parasites be found after the 4th day of treatment. Plasmochin compound has an even more rapid action for parasites were only found in 3 per cent of patients on the 3rd day and none later.

Conclusions.—(1) Plasmochin and its combination with quinine had a marked curative action on the chronic cases of benign tertian malaria treated. The average relapse rate was only 24 per cent amongst 86 patients. (2) Plasmochin compound gave better results than plasmochin only. Amongst 20 patients treated with this compound on 28 days no relapse was recorded in the 18 patients who completed observation. (3) The toxic symptoms make it necessary, in our opinion, to have a daily medical inspection of all patients undergoing treatment. (4) The length of the treatment, the alarm produced in the lay mind by the toxic symptoms and the necessity for daily medical inspection make it unsuitable in its present form for mass treatment in an uneducated tropical population.

D. Miscellaneous Preparations

Peracrina 303 was tried on a small series of patients. Parasites both sexual and asexual were found to persist in the peripheral blood of a large number of patients for long periods even while the maximum dose of the drug was being taken. Febrile relapses which required quinine treatment were liable to occur and the length of treatment was long and indefinite. The treatment does not seem capable of practical application (Sinton, Bird and Orte, 1927).

Smalarina Cremonese was also tried in a few cases and the disadvantages found were somewhat similar to those of peracrina.

The Production of a Clinical Cure

A. *Cinchona Alkaloids*.—The average duration of fever in 1,127 British patients suffering from chronic benign tertian malaria was 0.31 days. Only 2 patients, both under quinidine treatment, had fever after the 3rd day.

Our experience of benign tertian malaria has been that if fever lasts more than 3 days after the commencement of treatment with any of the cinchona alkaloids some complicating factor is almost certainly present. This is always provided that appropriate doses and methods of administration have been employed.

Over 600 patients were treated primarily with 30 grains of quinine daily in solution to reduce the temperature to normal. We have never seen either febrile or parasitic relapses amongst these patients during the period while an after treatment

of 10 grains of quinine daily in solution was given and retained Amongst over 1,500 patients suffering from benign tertian malaria, treated during the last 5 years, we have not been able to find a single one which showed quinine resistance although very many of these patients came with histories of having this condition

Amongst 1,241 British patients the splenic index fell from 47 per cent to 7 per cent with one month of continuous treatment with the cinchona alkaloids The reduction observed in the alkali series was greater than in the other cases

B *Stovarsol and Plasmochin* – The average duration of fever after treatment with these drugs was longer than after the cinchona alkaloids but when given in combination with quinine the duration was almost as short as with these alkaloids

Summary

(1) The best results in the production of a permanent cure in malignant tertian malaria were obtained with the quinine and alkali treatment

(2) Plasmochin seems to have a rapid destructive action on crescents

(3) Fresh infections with *P vivax* appear to be more easily cured than chronic ones

(4) The relapse rate in chronic benign tertian malaria after various treatments with the different cinchona alkaloids was about 60 to 70 per cent

(5) Plasmochin compound has produced a very high cure rate in chronic benign tertian malaria but the present form and dosage does not seem suitable for mass treatment in the tropics

(6) Both stovarsol and plasmochin cause a rapid disappearance of *P vivax* from the peripheral blood

(7) Stovarsol and plasmochin mark a distinct advance in the treatment of chronic benign tertian malaria

(8) At present the most hopeful line of research in the treatment of benign tertian malaria is the discovery of a drug like stovarsol or plasmochin but with a quicker action in the production of a permanent cure and a lower toxicity

References

Sinton, J A (1926) *Ind Jour Med Res*, Vol XIII No 3 pp 565 and 579

Idem (1926) *Ibid* No 4 p 895

Idem (1926) *Ibid* Vol XIV, No 1, p 227

Idem (1926) *Ibid* Vol XV, No 2 p 287

Idem, with Bird and Eastr (1927) *Ibid*, p 227

THE ACTION OF QUININE ON THE MALARIAL PARASITES

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IN 1921, King and Acton showed that, when a large dose of quinine, i e , a gramme of the anhydrous base was taken by the mouth, the concentration in the circulating blood did not attain a stronger solution than 1—150 000 In 1922 Acton found that quinine behaved differently on the *Paramœcium caudatum* when placed in an acid or alkaline substrate, thus quinine base was 10 times more powerful at a pH of 8 than at a pH of 6 Sinton (1924) also confirmed this enhanced action of quinine clinically by giving alkalies in the form of sodium citrate and sodium bicarbonate He stated that the enhanced action was produced by reducing the temporary acidosis in the blood Acton and Chopra (1926) working on this point, showed that by increasing the degree of alkalinity in the intestines, there was a greater diffusion of the quinine into the circulating blood, and so the concentration attained in the blood was higher when alkalies was administered before or with the quinine They also noted that the concentration of quinine was greatest in those mesenteric vessels coming from the gut where the quinine was being absorbed, and this concentration was greater than what occurred in the circulating blood, hence explaining the high cure rate of quinine in malignant tertian infections when the main site of sporulation coincided with the maximum concentration of quinine in the blood In 1919, Acton, Curjel and Dewey pointed out that, of the alkaloids of cinchona, quinidine appeared to be the most powerful in its action on the malarial parasites Since then we have tried this drug on the immediate cure rate of malaria, and found that although the drug was very powerful, it has a marked depressant action on the heart, particularly when the cardiac muscles were enfeebled by fatty degeneration or debilitating diseases We found that the quinidine was more rapidly absorbed from the gut and attained a greater concentration than quinine, but on the other hand the concentration

in the peripheral blood was less than quinine, indicating that the bulk of the alkaloid was absorbed by the internal organs In perfusion experiments on the heart, we found that quinidine was absorbed much more by the heart muscles than quinine Its toxicity can, therefore, be explained by its greater rate of diffusion, so that the concentrations attained are greater than quinine, whilst the heart muscles can absorb much more quinidine, as the concentration of quinine in the circulating blood never can attain such a strength that one would be able to kill every parasite in the body by a single dose or injection The drug has to be given over fairly long periods, three weeks or more, so that there must be only a partial destruction occurring with each cycle Moreover, we know from clinical experience that, if quinine is given some hours after the paroxysm, it very frequently fails to prevent the next attack, showing that when the parasites have matured and penetrated the red blood cells, the drug cannot diffuse through the erythrocyte membrane and reach the parasites King and Acton (1921) showed that the proportion of quinine in the red blood cells and serum was about equal and if the erythrocyte membrane allowed quinine to diffuse through it we would have expected more quinine in the red blood cells than in the serum Therefore it was necessary for us to study the effects that would be produced by sub lethal concentrations of quinine on lower forms of life With the *Paramœcium caudatum* we found that, if the pH of the culture was about 8, the certain lethal concentration was 1—35 000, but when the concentration was more dilute, i e , 1—120,000 out of the original 40 individuals that were inoculated in the culture only 10 were living at the end of 10 days, showing that multiplication was hindered and the death rate was greater than the rate of multiplication The effect on multiplication was seen up to a dilution of 1—250,000 At a dilution of 1—500,000 the quinine appeared to stimulate the rate of multiplication The details of the experiments are given in the Table below —

TABLE I

The effect of alkaloids on reproduction in 30 c cs of culture which contained the following dilutions of quinine, and was inoculated with 20 cm of paramœcium culture containing 46 organisms

	03	02	01	009	008	007	006	005	004	003	002	001	Mgs of an hydrous base
Quinidine				2	4	2	50	2	20*	100	100	200	No of organisms
Quinine		1	10	6	20	20	50	100	200	200	200	200	Ditto

Control = 200 organisms * in each field

Certain minimum lethal dose for 01 for Quinidine = 100 000
03 for Quinine = 35,000

Point up to which reproduction was hindered ·005 for Quinidine = 1—200,000
·003 for Quinine = 1—120,000

TABIF I—*concld.*

	03	02	01	009	008	007	006	005	004	003	002	001	Mgs of hydrous
Quinidine								150	300	6 450	18 000	7 500	No of organ
Quinine			300	450	300	2 100	2 700	1 800	6 900	6 000	7 950	8 100	Ditto

Control 6 450 organisms in 30 c cs
Certain minimum lethal dose in 12 days = 006 for Quinidine = 166 666
= 02 for Quinine = 50 000

It is known that the action of quinine in sub lethal concentrations produces paralysis of the movements of lower forms of life This paralysing action takes place before the protozoa is killed outright, it can be seen by using dilute concentrations of quinine on paramœcium One will first notice that the paramœcium becomes less active and finally the movement of the cilia ceases so that they come to rest at bottom of the vessel A few struggling attempts are then made by the protozoa to crawl along the bottom and finally they round off and die at death some change can be seen to take place in the protoplasm We consider that the quinine acts in a similar way on the malarial parasites because the concentration attained in the circulating blood is insufficient to kill the malarial parasites outright In these sub lethal concentrations between 1—120 000 and 1—250 000 quinine paralyses the movements of the young trophozoites that are adherent to the erythrocyte membrane The parasites owing to this loss of *amœboid movement fail to penetrate the envelope of the red blood-cells in order* to get its food The sluggish parasites are swept off the face of the red blood cells by the friction of the blood stream and failing to get inside the red blood cell, die later on from starvation in the spleen and other internal organs The parasites appear to be caught up in the splenic reticulum and destroyed by cytolysins produced by the reticular endothelial tissue and not by leucocytes (Knowles and Acton 1923) In malignant tertian infections, when the young trophozoites are extremely active and are seen adherent for some time to the face of the red blood cell the quinine can therefore exert its maximum action on these young forms Moreover sporulation occurs mainly in the deeper vessels of the mesentery etc where the concentration of the quinine is at its highest, therefore the cure rate of quinine is the highest in this infection

In conclusion we may say that the quinine molecule is more diffusible in an alkaline than in an acid substrate It attains a concentration in the blood which is probably sub lethal to the parasites In sub lethal concentrations the quinine hinders the movement of these parasites, so that they fail to reach their food supply On more mature forms of the trophozoites it probably hinders reproduction by

the formation of a smaller number of merozoites The young parasites that are adherent in a semi torpid state on the red blood cells are swept off by the friction of the blood stream They lose their food supply which they get from the red blood cells and die of starvation in the tissues of the spleen, etc The parasites are digested by cytolysins which are derived most probably from the reticular endothelial tissue

REFERENCES

ACTON CURJEL and DEWEY (1919 20) The diagnosis and treatment of benign tertian and malignant tertian fevers. *Ind Jour Med Res* Vol VIII

ACTON and KING (1921) The nephelometric estimation of quinine in the blood *Biochem Jour* Vol XV, No 1

ACTON (1921) The behaviour of *Paramœcium caudatum* towards the cinchona alkaloids *Ind Jour Med Res* Vol IX No 2 Oct

Idem with CHOPRA (1925) The concentration of quinine in the circulating blood *Ibid* Vol XIII No 1 July

KNOWLES ACTON and DAS GUPTA (1923) A note on spleen puncture findings in malaria *Ind Med Ga* Vol LVIII, No 5 May

SINTON (1925) Studies in malaria with special reference to treatment. *Ind Jour Med Res* Vol XIII p 759

EFFICIENCY IN MALARIA TREATMENT THE MERITS OF SILVER SALVARSAN

BY

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(1) *Introduction*

SIR PATRICK MANSON in his invaluable manual already stated that 'there is great difference of opinion and practice about the dose of quinine' Recent congress discussions (for instance, Fruit Co , U S A , 1924 , Malaria Congress, Rome, 1925) have once more shown how far from agreement international medical opinion still is in questions of first importance concerning the treatment of malaria I therefore thought it justified to draw the attention of the present congress to points fundamental in my view for the efficient treatment of one of the most important of tropical diseases

(2) *Dosis Efficiens of quinine pro die*

If the high value of combined quinine arsenic treatment seems to be well established and generally admitted, there still subsists much diversity of opinion as to proper doses and best way of administration of those drugs On one side very considerable doses *pro die* have been strongly recommended, especially during the great war Let me mention only the well known scheme of treatment given under direction of Sir R Ross for chronic relapsing cases not less than 60 grains *pro die,* half of which intramuscularly during 12 days, the same dose *per os* during the following 12 days By French doctors working in Macedonia, 30 grains (Ravaut) and 45 grains (Abrami) *pro die* used to be regarded as *minimum* dose In practice, the majority of medical practitioners, especially in the east, seems to find 15 to 20 grains *pro die* quite sufficient as an average dose The latter opinion was prevailing on the Rome Malaria Congress (1925), where James declared 10 grains twice daily, the suitable average dose for routine treatment Schuffner states to agree herewith 'in general' The most striking fact, however, i e , the fundamental relation between *body weight* and *dosis efficiens pro die* of quinine, seems to have been—if not overlooked—very badly neglected, as well in all manuals and treatises known to me, as upon congress discussions,

Strange to say, the evident necessity of *increasing* according to body weight the *dosis pro die* of a drug like quinine so quickly eliminated from the blood has never been established yet (as far as I know) in plain figures Independent of the gravity of the malarial infection one average dose of say 15 grains (1 gramme), of quinine hydrochloride in 24 hours whilst checking the attack successfully with patients of body weight below 60 kg (*ca* 10 stones *) might prove utterly inefficient with people weighing more than 70 kg (*ca* 12 stones) We therefore would invite the congress to figure out *a scale* able to give useful directions in the practice of quinine treatment (especially for routine treatment) and we beg to propose the following figures as a base for attack treatment —

Scale figuring relation between *body weight* and *dosis pro die* required of quinine hydrochloride —

below 8 stones (50 kg)	15 to 20 grains (1 to 1½ grms)
between 8 to 12 st (50 to 75 kg)	20 to 30 grains (1½ to 2 grms)
more than 12 st (75 to 80 kg)	30 to 45 grains (2 to 3 grms)

These figures to be taken as an average base for attack treatment, i e , during the fever and during at least 4 to 5 days after defervescence Perfect absorption secured the whole of the dose may be given *per os* In the majority of the cases in practice the safe way will be *to inject at least one half* of the daily dose intramuscularly This duly stated we shall not discuss the other (not less essential) points of successful dealing with malarial patients during the common attack and in the various clinical and ætiological forms We beg to draw attention to a few special perhaps less known points —

(3) *Intermittent quinine arsenic treatment*

If systematically applied in early attacks (i e alternant 2 arsenic days' after 4 to 6 quinine days) it is able to reduce in ordinary tertian the percentage of relapses For routine treatment we strongly recommend the hypodermic use of 10 per cent sodium cacodyl 5 c cs once a day as active and economic Following Ravaut we think the principle of intermittance most valuable in avoiding the phenomena of quinine resistant endless relapsing cases due probably to the accustoming action of prolonged uninterrupted use of quinine In private practice we find salvarsan intravenously very useful and suitable to realize the combined treatment The recent vogue of the older organic (pentavalent) as compounds (like stovarsol tryparsamide treparsol) mainly introduced by Marchoux, recommended as very active *per os* but only in tertian infection seems to be very promising for use on a large scale as an ambulant combined cure Marchoux's experience of stovarsol acting exclusively upon tertian parasites should not, however, be extended to a bivalent as compounds like salvarsan

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* 10 Stones = 63 kg

(4) *Silver-Salvarsan and Neo-Silver Salvarsan*

I am glad to insist on the *strong gametocide action* of *silver salvarsan and neo-silver salvarsan* given intravenously in *all* forms of malaria, and especially with crescent carriers as I have been able to experience in Sumatra ever since 1920, and to publish first accounts of results in 1922 and 1923 The dose 0 3 to 0 4 grm. intravenously reduces the number of crescents in the peripheral blood enormously within 4 to 6 hours In favourable cases complete destruction of the crescents is obtained *definitely* as proved by monthly controlled cases Resistant gametes (after the first silver salvarsan injection) often disappear after a second injection More resistant cases are still liable to be 'cleaned by combined intravenous cure of 6 to 8 grams quinine bihydrochloride and silver-salvarsan intravenously This fact is of great epidemiological interest, in my view, in that the bulk of carrier cases is liable if not to be freed, to have by one single silver salvarsan injection the mass of gametocytes enormously reduced The ordinary salvarsan may give comparable results I personally far prefer silver salvarsan as more active, more stable against climatic influences, and consequently giving less apprehension for toxic accidents

(5) *Arsenic, Iron and other Tonics*

Next to arsenic, iron and other tonics, I would like to call your attention to *metallic iodine* as valuable and interesting, in malaria treatment One sees wonderful effects upon certain chronic cases, especially upon the enlarged spleen *We give it per os as a 5 per cent tincture three times a day*, 5—10—20 and more guttæ We believe there are cases answering much better to iodine than to the traditional arsenic iron cure, as far as given *per os* We are actually using iodine also intravenously (as weak Lugol solution) combined with silver salvarsan where it lessens the shock at the same time improving the therapeutic effect

(6) *Adrenaline Test*

If still I may retain your attention let me in short explain what we call the adrenaline test

Suppose a malarial splenomegaly of the third degree (spleen nearly reaching navel), 1/10 c c of adrenaline (P D & Co) (*i e* , what remains *in injection syringe* after one injection) is given with a few (3 to 5) c cs intravenously in the right vena cubiti The right hand controls the enlarged spleen Half a minute (or sooner) after the intravenous injection of adrenaline in most of the cases one feels the spleen getting smaller and smaller, reaching not seldom the second and the first from the third degree That is to say, the spleen, under direct adrenaline action, undergoes strong contraction, diminishing its volume like a sponge pressed in the hand We have used the adrenaline test in some 100 cases, without any unwanted by effect , on the contrary, the adrenaline does much good in chronic as well in severe acute cases As the result of our investigations we may suggest the

following *conclusions* enlarged spleens answering to the adrenaline test with strong contraction are liable to regress (diminish) under internal adequate treatment (arsenic, iron, iodine, strychnine etc) fairly well On the contrary, spleens not or weakly answering the adrenaline test may be looked at as sclerosis lienalis mostly incurable by medical treatment and consequently cases for eventual splenectomy With regard to the adrenaline test the so called provocation proof ' we have never had any positive result in our cases We first heard about adrenaline acting upon the spleen in the interesting paper by Messrs Pagniez, Coste et Escalier

REFERENCES

JAMES S P (19?5)	Trans Malarial Congress Rome
SCHUFFNER	*Ibid* (Abstract)
MARCHOUX	*Annal Inst Past*
PAGNIEZ COSTE et ESCALIER (1925)	Etude sur la contractilite de la rate, *Presse Medicale*, No 99

SOME GRAVE CASES OF MALIGNANT TERTIAN MALARIA TREATED WITH INTRAVENOUS INJECTIONS OF QUININE

BY

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In this province one has to deal with large numbers of cases of malarial fever At times it breaks out in epidemic form in rural areas towards the end of the rainy season

The majority of the cases does not put up any problem in their treatment Simple oral administration of quinine checks acute attacks Sometimes one meets with types of cases particularly in sporadic forms, in times and places not notorious for this disease, which tax the utmost skill and judgment of medical men for diagnosis and treatment They constitute one of the emergencies of medicine, that is unless these cases are rapidly brought under the control of the specific drugs the case is lost

In the oral method of quinine administration it takes at least 3 hours for the drug to be fully absorbed as has been worked out by pharmacologists like Dixon and others Moreover, the state of the gastro intestinal and hepatic tracts under these conditions does not allow of its ready entry into the blood Oral methods cannot, therefore be relied upon in these cases

The intramuscular route is also useless for the rapid mobilization of the drug. It is much slower than the oral method, as has been shown repeatedly by numerous workers, although its action lasts longer in the system than other methods

In grave cases of malaria, certain vital parts of the body are the sites where the parasites sporulate and tend to choke the free circulation by the formation of parasitic emboli and thrombi In this way the functions of these vital parts are disturbed and life is put to danger

One has, therefore, to bring the quinine to these sites as rapidly as possible and in sufficient concentration

The intramuscular method is ordinarily sluggish Moreover, the weakness of the heart and fall of blood pressure in these cases due to shock lowers the rate of entry of the drug to the system

Time and again one has witnessed cases of the cerebral types of malaria ending fatally in spite of their being treated with repeated intramuscular injections for days together. The rectal and inunction methods are only of academic interest.

Now comes the question of intravenous quinine. It is the most rapid method of introducing the specific drug to the blood. But intravenous quinine in doses recommended in text books brings down the blood pressure very much. In one or two instances I am aware of the collapse was instantaneous, so much so, that there was scarcely any time to take out the needle before life was extinct. Besides the fall of blood pressure one witnessed considerable respiratory distress. In these emergency cases, 8 to 10 grains were used for the single dose.

On occasions 10 to 15 grains have been injected intravenously in a single dose with impunity for refractory types of malaria during the afebrile period. In such cases the venous route was chosen to obviate the induration and pain, and rarely suppuration at the injected site.

Very dilute solutions of quinine such as 200 to 300 c cs of normal saline containing doses like 8 to 10 grains of quinine, with the idea of flushing the blocked and sporulated vessels and picking up the blood pressure have not been found to be free from risks. The fall of blood pressure in malaria, unless it is in choleraic cases, is not due to loss of fluid, but to a condition allied to shock. Fluid introduced into the vessels does not improve the situation, but rather makes it worse by bringing about cardiac distress ending in fatal pulmonary œdema. Some workers have contended on theoretical grounds that 10 to 15 ounces of fluid can be easily accommodated by the system, but they forget that it is, as it were, the last straw on the already overburdened camel's back.

The writer has attempted to face the situation in a different way. Instead of using the maximum dose all at one time it has been given in a fractional method. The same dose of 8 to 10 grains was divided into 3, 4, and sometimes 6 separate doses in the 24 hours given at intervals depending on the severity and gravity of the constitutional condition as made out by the state of consciousness and the circulatory and respiratory states.

Some of the typical cases of the series are as follows —

Case 1. Child H. M. admitted to the Children's Ward of the Carmichael Medical College 12th September, 1924. Age 14 months, fever four days, frequent convulsions for the last 2 days. History of fever off and on for the last 6 months. Blood showed heavy infection of malarial rings in every field. Quinine bihydrochlor., grains 3 injected intramuscularly showed an eschar at the site after 12 hours. No improvement of condition on the following morning, pulse uncountable, heart sounds very feeble, no distinction between 1st and 2nd sound, temperature 101° patient comatose, no conjunctival reflex, convulsions very frequent. Injection of quinine bihydrochlor. grain 1½, from Wellcome's ampules, mixed with 2 c cs of normal saline into the external jugular vein given. In half a minute another severe convulsion occurred and the pulse was lost. Ice bag over head, warmth to the rest of the body and bromide of soda grains 2, per rectum, controlled the situation. Bromide was repeated every 6 hours. On the following day the child was conscious, convulsions very few. Dose of intravenous quinine, ½ grain, was repeated with intramuscular pituitrin 2 minims, in 2 c cs of saline. The half grain dose of quinine was repeated at 6 hourly intervals, i e., 4 in the 24 hours. On the following day the patient was conscious, free from fits and fever

Afterwards quinine was given in one grain doses, *per os*, t d s and the patient was discharged cured

Case 2 H Bose was seen on 4th November, 1924, at 5 P M for high fever, painful and frequent stools, full of mucus and blood There was slight jaundice, spleen, 2 fingers breadth below costal arch Repeated attacks of fever off and on checked by quinine In the morning he had taken 10 grains of quinine of his own accord At the time he came under observation, nausea and *vomiting was constant and distressing* Nothing could be retained temperature 101°F , pulse 110, very soft regular Slide was taken and, without waiting for the report, 3 grains of quinine were given intravenously in 5 c cs of saline The blood showed a heavy infection of malaria At 12 o'clock the same night he vomited blood and passed bright red bloody urine which showed intact r b cs Another dose of grains 3 intravenously was given and on the following morning the temperature came down to 100°F , with diminution of blood in the vomit and evacuations The urine was clear Intravenous injections of quinine in 3 grain doses were given at 6 hourly intervals, 3 times a day, until there was sufficient improvement to resume oral quinine

Case 3 R Poddar seen on 15th September, 1924, repeated attacks of irregular fever for four months, big spleen Present fever 7 days, took quinine of his own accord, temperature 102°F , bright red urine, showing intact r b cs Quinine, in 3 grain doses intravenously, at 6 hourly intervals, t d s ended in recovery Blood examination malaria parasites positive

Case 4 A S , age 55 seen for profuse watery stools, stoppage of urine, very feeble pulse, husky voice on 16th October, 1925 Gave the history of the trouble preceded by fever of 4 to 5 days duration At the time of examination surface temperature was subnormal but rectal temperature was 103°, spleen enlarged, evacuations watery, yellow Clinically the case looked like one of algid malaria Blood, sp gr 1060 Slide taken and at once normal saline, one pint, with quinine, grains 3, was given Slide confirmed suspicion of malignant malaria In 6 hours the condition of the patient had improved and another dose of quinine, grain 3 in 5 c cs of saline, was injected intravenously In addition, normal saline in 4 ounce doses was injected per rectum every two hours There was persistent hiccough Urine started after 8 hours After three daily intravenous injections of quinine in 3 grain doses the patient was cured

One could multiply instances like the preceding but they are almost of the same types There were two deaths in a series of 48 cases

Finally before closing this paper I would like to bring to your notice a case of malaria of the acute cardiac type

Dr S C P , aged 30, seen on 12th October, 1927 Fever 7 days of a very low intermittent type Past history in the sound health for the last 7 years, no spleen, no jaundice On the day seen by the writer patient perfectly conscious, temperature, 100°F (axilla), 102 4°F (mouth), pulse 130, very irregular, cardiac rate above 152, uncountable, respiration, 32, blood pressure 110 systolic Euquinine, grains 5, had been given twice on the previous day Physical examination negative Blood examination showed heavy infection with rings, r b cs 5 000,000 , w b c 9,670 , polynuclears, 66 per cent , S M , 14 per cent , L M 20 per cent

Quinine, grains 3 in 25 c cs of 12½ per cent glucose (Merck's ampules), injected intravenously After 10 minutes patient became very restless and dyspnœic , respiration rate, 64, laboured, blood pressure, 90 (systolic) Atropine, 1/100 grain injected subcutaneously as well as pituitrin ½ c c , dyspnœa relieved

13th October, 1927 Patient feeling very comfortable in the morning Heart and pulse still dissimilar vomiting incessant, mind quite clear, temperature 100°F In the evening he became maniacally delirious, pulse very feeble almost imperceptible One dose of quinine, grain 3, was given by mouth but at once rejected There was diarrhœa and tympanites Patient was injected intramuscularly with grains 5 of quinine bihydrochlor

14th October, 1927 Patient completely unconscious in the morning Blood pressure 125, pulse 100, Kernig s sign positive, neck stiff, mouth could not be opened No food, no medicine by mouth Five per cent liquid glucose with ½ per cent soda bicarb in 4 oz doses, every 3 hours per rectum Quinine, grains 3 with 25 c cs of Merck s 12 5 per cent liquid glucose intravenously, b d , 6 leeches applied 3 on each mastoid, at 8 P M

15th October, 1927 Pulse 92, regular heart, corresponding temperature, 99 8°F (axilla), 101°(rectum), respiration, 28 blood pressure, 125 85 (systolic—diastolic) look intelligent neck stiff, cannot talk, chest free, abdomen flaccid cannot swallow, legs extended could not be flexed

R b cs , 4,800,000 , w b c , 5 599 , polymorphs , 82 per cent , S M , 12 per cent, L M 6 per cent H B , 80 per cent , malaria parasites negative in thick film

Quinine, grains 3, in 25 c cs glucose intravenously, b d Lumber puncture done = 20 c cs of clear fluid under pressure, c.s fluid examined —sugar positive , cells mononuclear, 2 3 cells per c mm , no organisms on smear or growth

Pulse 84 , respiration 28 temperature 100 4°

16th October, 1927, 10-30 A M —Temperature 99 4°F pulse 88, blood pressure 110 (systolic) , patient conscious , taking interest in surroundings , no stiff neck, no babinsky

7 P M —Temperature 100°F pulse 100 , patient could keep down nourishment quinine grain 3 b d intravenously

17th October, 1927 Patient conscious morning and evening temperature, 98°F , pulse regular 108, heart corresponding could take quinine by mouth grains 3, t d s Convalescent and quite well again in a month

My thanks are due to the hospital authorities and doctors who very kindly permitted me to use the notes of their cases

DISCUSSION

Prof J W W Stephens (Great Britain) (*a*) Lieut Col James' data show that about 25 per cent of patients bitten by infected mosquitoes do not develop attacks early or late We can only conjecture what happens to the injected sporozoites but we know that foreign bodies introduced into the circulation are filtered out by the spleen, liver and more especially by the lungs

(*b*) ' Not everyone who receives a dose of sporozoites develops an appreciable malarial attack within the usual incubation period of the disease These cases appear to be of the same kind as those we sometimes see in clinical practice, viz , those patients who have their first attack of malaria on returning to England from the tropics

(*c*) *Immunity* —The results suggest that it may be possible and practicable to inoculate at home against malaria in the tropics

(*d*) *Absence of parasites* —A number of the charts show non parasitic temperature curves which cannot be distinguished from those with parasites and there can be little doubt that the former are malarial though it is not possible to bring forward absolute proof They place us in considerable difficulty and we shall go astray—how often I do not know—if we say ' no parasites, no malaria ' There seems to be no absolute necessity

that the same number of parasites exist (somewhere else than in the peripheral blood) as in the parasitic cases ? The only certain knowledge we have of the action of quinine is that it causes parasites to disappear We do not know that it destroys them.

Dr C D Esch (Central Provinces) We appreciate what we have heard from the honourable gentlemen who have given us such excellent papers this morning

Could one of these men kindly give us something in the way of an effectual and safe treatment of malaria in pregnant women ? Col James mentioned a case where

one patient failed to demonstrate malarial fever, after being infected when he was living an active life in the cold weather while another patient with the same infection who was kept in bed in a warm room showed manifestations of the disease Another case infected failed to demonstrate any symptoms when she was kept quiet but when she was allowed to move about freely developed as a typical case of malarial fever Would Col James please explain this apparent discrepancy ?

Dr S B Surti (Hyderabad State, B India) It would be presumptuous on my part if I entered into comments on the very able and interesting papers read, but I find that the practical difficulties that one comes across in administering large doses of quinine have not been dwelt upon in any of them , for example, if I give even 5 grains of quinine more than once to my patients, they commence trembling, and complain of palpitation and are not able to follow their daily routine of work Quinine by itself in the treatment of malarial fever in my hands has proved absolutely useless and is likely to do more harm than good as it decidedly acts deleteriously on the heart The role played by carbolic acid in the treatment of malarial fevers has not received enough attention at the hands of the medical fraternity and my usual routine of treatment in cases of malarial fevers is as follows —As soon as a patient is brought to my notice I prescribe a mixture containing 5 grains of cinchona febrifuge 3 minims of acid carbolic, with 10 minims of ipecac and about a drachm of magnesium sulphate thrice a day, even if the patient tells me that his bowels are regular, for, on a sluggish liver and constipated bowels quinine does not seem to have any desirable effect If the fever does not come down to normal within 24 hours, I as a matter of routine, give 10 grains of quinine bihydrochlor intramuscularly, and, if this measure fails to bring down the temperature then I administer sulfarsenol No 2 a substance akin to salvarsan I have also found sodium cacodylate a very useful preparation in cases of malaria I inject ¾ gr in 1 c c of distilled water, subcutaneously, continuously for 7 days and find it has a marvellous effect in checking the fever

Just before I came to Calcutta I gave plasmochin in 3 different cases in which the above line of treatment had failed to bring about the desired effect and, in each case, I found this drug giving excellent results, only 6 tablets bringing down the temperature

other potent drugs for administration in cases of malarial fever without producing symptoms of quininism

Lieut Col C A Gill I M S (Punjab) Thought everyone realized the extremely *important nature of Col James paper as a contribution to the epidemiology of* malaria Hitherto Europe had looked to the tropics for every advance in respect both of the epidemiology and the treatment of malaria, but the Wagner Janregy method of treating G P I had placed European malariologists in a better position than tropical workers for carrying out certain types of investigation We, in this country, never saw cases of G P I but even if they were available, the possibilities of malarial infections would always have to be taken into account Col James had expressly stated that his observations in England were not necessarily applicable elsewhere and he (Col Gill) wished to emphasize this point because it seemed to him that the utmost caution must be exercised

in basing generalizations upon the results of these experimental infections in England The results obtained by Col James were indeed diametrically opposed in many important respects with those obtained by the speaker in India He thought everyone must have been greatly impressed with the ease with which apparently severe infections with the benign tertian parasite were controlled by means of one small dose of quinine Col James had also concluded, as the result of experiments in England, that most people are refractory to malaria and that most Anophelines (*A maculipennis*) are bad transmitters He (Col Gill) could not reconcile these conclusions with his own observations and experiments When one had seen the whole population over wide tracts laid low by malaria during an epidemic, it was difficult to believe that most people were refractory to infection He mentioned his own case when, as the result of a single bite of one infected mosquito, he contracted malaria on the 16th day Then again, many experiments with many species of Anophelines conducted over a series of years in the Punjab had led him to conclude that all the common carrier species in the Punjab, even as the result of a single feed upon a suitable case of malaria were *remarkably good* transmitters He ordinarily obtained positive results in 50 to 100 per cent of cases in feeding experiments, but the only completely negative result that he could recall at the moment was obtained whilst working in London School of Tropical Medicine in 1923 when a batch of 40 *A maculipennis* which had been fed upon a patient with a heavy infection, all proved negative Subsequent inquiry, however, elicited the information that the patient, a sailor, has been given salvarsan some 8 hours previous to the time of feeding the Anophelines This observation led him to consider whether arsenical preparations had been given to any of Col James' patients It must be remembered that many of these patients were syphilitics and it was therefore probable that they were also being treated by salvarsan or by other arsenical drugs as well as by malaria He asked Col James for information upon this important *point* It must also be remembered that if, as is assumed the malaria toxin is *inimical* to the parasite of syphilis it is conceiveable that *T pallidum* may exercise a similar influence upon the malaria parasite Be this as it may, unless Col James could assure us that his patients were not in receipt of any other treatment except artificially induced malaria, it would be impossible to regard his experiments, from the epidemiological or indeed from the therapeutic point of view, as clean experiments Furthermore, the study of the influence of climatic conditions upon malaria suggested that conclusions based upon observations conducted at high altitudes or in cold climates upon the influence of the cinchona alkaloids upon malaria were not necessarily applicable everywhere and it would, therefore, seem to be expedient at present to regard the conclusions reached by Major Sinton (and by Major Acton) as applicable only to the effect of their drugs under the climatic conditions prevailing at Kasauli and Dagshai respectively, i e , at altitudes of between 4,000 to 5 000 feet above sea level

He was, however, chiefly concerned with the epidemiological side of the problem and more especially with the conspicuous divergence between Col James and his epidemiological observations and laboratory experiments He again asked Col James whether his experiments were clean experiments, for he felt strongly that, unless they *were*, extreme caution must be exercised in basing conclusions upon them of general epidemiological significance

Dr S L Sarkar (Bengal) Under the auspices of the Indian Research Fund Association I had to carry out experiments with cinchona alkaloids on bacteria protozoa, as well as upon guinea pigs In the experiments upon guinea pigs, I found the cinchona alkaloids as cinchonine sulph, quinine sulph cinchonidine sulph, to have depressing effects upon the heart The only cinchona alkaloid which has not a deleterious effect upon the heart is quinidine sulph I have used the knowledge gained by the laboratory experiment in clinical practice in the following way —

Whenever I have found the heart to be weak, instead of giving quinine sulph alone, I take the dose of quinine sulph and add to it an equal amount of quinidine sulph, to keep up the antiperiodic property In this way the depressing action upon the heart is avoided The reduction effect upon the spleen is more marked when this combined salt is used than when quinine sulph is used alone Some obstinate cases of malarial fever yield readily to this combined drug when quinine sulph used alone has failed From my experience of using the drug I believe that it cures mild cases of kala azar, though I cannot be definite on this point, as confirmation of the results have not been made by bacteriological examination

Dr D P Williams (Assam) I wish to raise the question of the administration of quinine to pregnant women European and Indian, both from a curative and prophylactic standpoint, or rather, if I may be so allowed, to make an appeal to this distinguished body of malariologists to give a definite, authoritative and final pronouncement on a question that vitally affects medical officers in the East, especially those who are ploughing a lonely furrow in out of the way places It is a question that confronts us again and again where our responsibility is greatest The question naturally divides itself into two (1) Is there any medical objection whatsoever to administering quinine to a pregnant woman at any time during her pregnancy as occasion arises either from the point of view of the mother or the child, and (2) if there is any danger involved is it at all comparable with that incurred in allowing a malarial attack or repeated attacks to run their course uncontrolled by quinine? Personally not only do I hold strongly that there is no danger whatsoever in giving quinine right through pregnancy, if it is called for but that it almost amounts to malpraxis not to do so unless we have used every means in our power consistent with the dictates of humanity to overcome our patient's objections While quinine has no action on the pregnant uterus, except possibly in actual labour, even one single attack of malaria during pregnancy frequently ends in a tragedy I presume that some British gynæcologist in days long ago, himself being so taught by a pharmacologist, made the statement that quinine was an abortifacient This statement copied from testbook to testbook, *secundum artem*, is still repeated by young medical officers on arrival in the East, ladies repeat it to ladies, mothers to daughters, neighbours to neighbours until now it has been accepted for many years as an article of faith, even of sex loyalty, to their ultimate sorrow and the despair of the doctor The time at my disposal does not allow me to give the evidence for the statement that quinine is innocuous in pregnancy, but to me the evidence is cogent and final All of you who have had to deal with hundreds of cases of the profound anæmias of pregnancy have this evidence In no case in my experience has quinine, even in colossal doses any more than any other drug, had the slightest effect in an attempt to terminate

pregnancy Besides, it is sold openly in all druggists shops in the world The heaped-up tragedies of the effects of uncontrolled malaria in pregnancy are common knowledge On behalf of our patients and also on behalf of doctors, especially of young doctors working in distant provinces I venture to appeal to this body of representative malariologists to give to us an authoritative and final judgment on this question to which we can appeal and which we can quote in times of stress

Sir Malcolm Watson (Federated Malaya States) After a large experience of the disastrous effects of malaria on pregnant women had no hesitation in giving pregnant women quinine He knew of an estate where no living child was born for several years All women who became pregnant aborted He treated pregnant women with malaria exactly as he treated any other case of malaria

As a student he had been warned by his old teacher, Sir William Gairdner, of the danger of large doses of quinine in non malarial fevers like typhus, typhoid, etc Sir William emphasized that 45 grains of quinine produced grave shock and might kill Guided by this teaching he had rarely given more than 20 to 25 grains in the 24 hours, he was glad to hear that *modern* scientific observations went to show that very large doses gave no better results than the smaller ones provided the smaller ones were not as small as 2½ grains His own view had been for years that quinine was not a direct poison of the parasite

Major Sinton's work was important because they must learn the cheapest method of treating large numbers of people But for many, cost need not be considered, and what many patients wanted was a treatment that would be a practically certain cure

Major Sinton has suggested that the solution of the malaria problem might be a drug which could cure in three days They knew of something parallel A yellow fever patient could infect mosquitoes for only 3 days The disease ceased to be infectious in that time Yet even with this limited period of infection the Americans were almost driven out of Panama by yellow fever after fighting it for over 18 months The town was fumigated 5 times before they stamped it out

Mr R Senior White (Bengal) Col James has pointed out that the same strain of parasite will not cause more than two or perhaps three infections Has Col James tried to infect with the same strain using different carriers of which he has two other species than *maculipennis* available ?

Dr M C Murphy (Assam) Major Sinton appears to take a period of from six to eight weeks freedom from fever and symptoms as sufficient to establish a cure Col James states that relapse may occur after eight or nine months, a statement which contradicts this but which is borne out by clinical experience

Dr R J Gittins (Central Provinces) I wish to ask Major Sinton if he will enlighten us on what he considers to be the best form of cheap treatment for general hospital work among the poorer classes Further, I would ask Col James if his work has gone to show that in virgin cases of malaria the first manifestation of fever is in the form of a few days continuous fever, as indicated in a paper published in England early this year

Lieut -Col R Knowles, I M S (Bengal) May I say with what profound interest I listened to Col James' paper ? This question of individual resistance or susceptibility

to protozoal infections is one of the greatest importance In studying the history of medicine one may say that our knowledge with regard to any parasitic disease seems to pass through four phases The first is the one prior to the discovery of the parasite concerned Here diagnosis has to be based on symptoms and signs, and this period is, therefore productive of the great clinicians, such as Sydenham The second opens with the discovery of the parasite, and attention now becomes focussed on laboratory diagnosis The third period opens with the recognition that the soil is of equal importance with the seed It is this period which now seems to be opening up in our study of protozoal infections If we could only understand the underlying mechanism of resistance or susceptibility to protozoal infections our treatment of these diseases might become revolutionized There are probably all sorts of factors concerned in this problem of resistance to malaria, questions of blood sugar content, of endocrine activity and the like, and, speaking as a protozoologist, I would welcome the invasion of the domain of medical protozoology by the biochemists

The further period, as Sir Ronald Ross has long insisted, opens with our grasping the idea that quantitative studies of disease are of equal importance with qualitative ones, that we must evolve methods of studying and measuring the intensity of the disease in the individual as well as in the general population

Turning to the question of *how* quinine cures malaria, I think that evidence is now steadily accumulating that in these chronic protozoal infections the action of the drug is an indirect and not a direct one To give an example, it is quite common after a complete course of antimony treatment to still find a few residual leishmania in spleen puncture films Yet you discontinue treatment, and six months later the patient comes back to you in excellent health Hence I do not think it necessary to aim at the *therapia magna sterilans* which Major Sinton suggests What we want is to investigate and thoroughly understand the mechanism of natural immunity against, and of spontaneous cure of malaria and here the biochemist comes in It may be sufficient to scotch the infections and to trust to the natural powers of resistance of the body to get rid of the residual parasites and in this connection both Col James' and Col Acton's papers were of very great interest

Major J A Sinton I M S (B India) replied The discussion on the prophylaxis of malaria has turned mainly on the human and the mosquito factors, while the parasite factor has been almost entirely ignored It seems to me that *if* we could obtain a drug which would cure malaria in three days, we would probably have one solution of the malaria problem in our grasp—a solution which would be practicable in many, if not all, rural areas The fact that synthetic drugs have, at last, been discovered which have a definite action in malarial fevers is a very hopeful sign Further research along these lines should be pushed in the hope that a drug may eventually be discovered which will fulfil the essential points of an ideal treatment laid down in the paper

Sir Malcolm Watson has objected to the suggestion that such a discovery would prove one solution of the problem In kala azar, I understand that the antimony treatment is already playing an important rôle in the eradication of this chronic disease The comparison with yellow fever is not applicable, in my opinion because the economic importance of this disease depends largely on its high mortality rate, while the importance of malaria lies mainly in the great amount of sickness and debility

produced Even if such a drug did not eradicate the disease, it should have an enormous effect in reducing its economic importance

Several members have asked for a definite expression of opinion as to the best standard treatment to adopt It is regretted that no such definite opinion can be given, for, as indicated in the paper the effects of treatment differ with the type of parasite and with the chronicity of the disease in the case of benign tertian malaria It also depends on whether the patient will continue treatment until a permanent cure is produced or only until clinical symptoms are ameliorated

Dr Williams has inquired regarding the use of quinine in pregnancy My personal experience has been that quinine given in doses up to at least 20 grains daily by the mouth in combination with bromides has had no deleterious effects in this condition The opinion formed by me has been that more abortions etc are caused by untreated malaria than by quinine if indeed the latter has any such action, except when the disease has already stimulated contraction of the uterus

In reply to Col James and Dr Murphy with regard to the adequacy of an 8 week observation period after the cessation of treatment This was the minimum period during which we attempted to keep our patients under observation by blood examinations after treatment Numerous patients were observed for longer periods in this manner and the later clinical histories of many patients are available We believe that by this method it is possible to detect about 90 per cent of the cases which will relapse after treatment

Dr B Shaha (Bengal) replied —(1) Quinine bihydrochlor or quinine hydrochlor in 5 grain doses dissolved in a dram dose of spirit vin gallici once a day has been found to be very efficacious in the refractory types of benign tertian infection For prophylaxis it has been found to be very useful in the outbreak of epidemics (2) Ten to fifteen grains a day has been found to be very efficacious in cutting short an acute attack and curing it clinically The writer as a volunteer to the quinine excretion experiment of Col Macay then Major Macay in 1912 was unconscious for 12 hours after a single oral intake of 25 grains of quinine alkaloid.

Lieut Col S P James I M S (Retd) (Great Britain) replied In reply to Dr Esch the cases cited are examples in which warmth in the one case and exercise in the other seemed to have some influence in bringing on a clinical attack of malaria in infected patients I do not find anything contradictory in these results but I am unable to explain how these and other factors act In reply to Col Gill I readily acknowledge that some of the results of our laboratory work on the infection of Anopheles and of man and on the treatment of patients in England are apparently quite different from the results of experience in the tropics and in my paper I have expressed the view that a long series of local researches on the subject will be necessary in the tropics before final conclusions are reached In reply to Col Gill s question about salvarsan, I can assure him that none of our patients were being treated with that drug or other arsenical preparations prior to being given malaria therapy In reply to Mr Senior White we have not as yet, attempted to re infect with any other species of Anopheles than *maculipennis* Dr Gittins is correct in stating that during the first stage of a primary attack of pure benign

tertian infection the fever is quotidian not tertian I regret that I cannot share Major Sinton's opinion that an 8-week observation period after the cessation of treatment will reveal about 90 per cent of the cases which will relapse Recrudescences will be detected during that period but none of the cases of 'long relapse' which occur between the sixth and tenth month after the primary attack

RAPPORT SUR LES RESULTATS DU TRAITEMENT DE DIVERS ETATS DE PALUDISME PAR LA *SMALARINA* DU PROF CREMONESE

PAR

LE COL I FROILANO DE MELLO

Directeur des Services de Santé et Hygiène à l'Inde Portugaise

Wednesday Dec 7th, 2 to 4 P.M

INTRODUCTION

LA *smalarina* du Prof Cremonese est un composé de mercure et antimoine, synthèse chimique de nature colloïdale—dit l'auteur—très instable qui est très vantée par son auteur et quelques confrères italiens comme le traitement par excellence du paludisme *Traitement radical et immunisateur composé idéal* telles sont les qualifications qui lui ont été données par le Prof Cremonese La formule chimique de ce produit est $C_8 H_{13} O_7 N_4 Hg Sb$ il est livrée dans le commerce sous forme de comprimés dont l'emploi se fait per os de la façon suivante chez les adultes 1 comprimé le premier jour 2 le troisième 3 le cinquième et ainsi de suite prenant le médicament en des jours alternés et augmentant d'un comprimé chaque fois jusqu'à atteindre la dose de 16 comprimés le 31 ème jour ou soit un total de 136 comprimés

Contre indiqué à peine chez des brightiques pouvant être administré même aux bébés au dessous d'un an à doses réduites cela va sans dire son action curative a donné issue de le part de son auteur à des théories très intéressantes sur le mécanisme de la guérison du paludisme et sur l'action immunisante de cette drogue qui est tellement puissante *qu'il est difficile que de nouvelles infections paludéennes se produisent pour des mois et même au moins pour une année* (Cremonese 1925)

Pour compléter ce court aperçu sur la smalarina dont l'efficacité est telle que l'auteur peut *affirmer en toute confiance que tous les cas—des vieilles formes resistantes à la quinine chroniques etc —traités par ce composé ont cédé a son action en un temps plus ou moins court* il ne me reste qu'à signaler que la valeur *princeps* de cette drogue est dû au mercure déjà vanté par des anciens auteurs (siècles XVII à XIX) dans la thérapeutique du paludisme et tout à fait oublié par des malariologistes modernes et une valeur accessoire à l'antimoine *parce que* dit Cremonèse, *l'expérience m'a démontré l'utilité de ce corps comme coadjuvant de la thérapeutique paludéenne*

Essais Thérapeutiques

Sollicité pour faire des expériences sur ce produit, large et libéralement mis à ma disposition par les chimistes italiens j'ai voulu faire une série d'essais en les controlant par des recherches cliniques, hématologiques et parasitaires qui me pûssent donner des élements d'appréciation sur l'efficacité de smalarina

Aidé par mes éleves et par mes délegués, ceux ci exerçant dans des localités très malariennes et invités officiellement à collaborer dans cette enquête, j'en donnerai les résultats dans les tableaux à suivre non sans ajouter que dans les éssais de thérapeutique expérimentale nous devons nous attacher d'abord aux faits qui restent qu'aux théories qui sont souvent si fallacieuses et que dans l'infection paludéenne, lors qu'il s'agit de telles expériences le *test princeps* qui doit les orienter c'est évidemment la recherche de l'hematozoaire Si celle ci est positive, le paludisme n'est pas guéri que cela déplaise aux théoriciens, pour plus ingénieuses qui soient leurs conceptions Et si la recherche de l'hématozoaire est négative il faudra une prudente reserve pour formuler des conclusions, puisqu'il n'y a pas de malariologiste au moins parmi ceux qui travaillent aux tropiques, qui n'ait pas vu qu'il y a des paludéenns surtout chroniques, avec d'indoubitables symptômes de malaria fièvres irregulieres, splénomégalie, etc , dont l'examen du sang ne décèle pas souvent des plasmodies aux plus minutieuses recherches !

Passons donc a exposer mes resultats Dans chaque série on trouvera résumés les divers éléments qui plus détaillément seront publiés dans les *Arquivos da Escola Médico Cirurgica de Nova Goa* dans un prochain numéro

Index Bibliographique

Cremonese, G (1925) 'La Smalarina Cremonèse traitement radical et immunisateur du paludisme sans quinine Roma Casa' Ed F Mantegazza, p 17

Idem 'Malaria Vues nouvelles sur la doctrine et sur la thérapeutique' Rome Vol MCMXXIV, p 73

SERIE I

Malaria Chronique avec Récidives Pyrétiques

No	1	2	3	4	5	6
Histoire de la maladie	Fièvre quotidienne pendant 15 jours il y a 2 mois Récidives irregulières tierces ou quotidiennes	Contracté *depuis* 8 ans Type tierce à intervalles irreguliers	*Accès quotidienns* il y a 15 jours Apyrexie	Malade il y a *trois mois accès quo*tidienns pendant 3 semaines	Malaria tierce irregulière il y a quelques mois	Malaria contractée il y a 1 an Récidives quotidiennes et tierces irregulières
Symptomes actuels importants	Anémie teint subictérique Vecator Amèbes Ascaris Tricocephale	Nouvel accès typique ave frisson et sueurs	Nouveaux accès *Anémie* Subictère	Nouvel accès Légère anémie	Anémie légère	Nouveaux accès tierces typiques
Rate	Palpable	Non palpable	Non palpable	Non palpable	Non palpable	Non palpable
Numero total de comprimés de Smalarina	105	120	120	120	120	55

Essais Thérapeutiques

Sollicité pour faire des expériences sur ce produit, large et libéralement mis à ma disposition par les chimistes italiens j'ai voulu faire une série d'essais en les controlant par des recherches cliniques, hématologiques et parasitaires qui me pussent donner des élements d appreciation sur l efficacité de smalarina

Aidé par mes éleves et par mes délegués, ceux ci exerçant dans des localites tres malariennes et invités officiellement à collaborer dans cette enquete j en donnerai les résultats dans les tableaux à suivre non sans ajouter que dans les éssais de thérapeutique expérimentale nous devons nous attacher d'abord aux faits qui restent qu'aux théories qui sont souvent si fallacieuses et que dans l infection paludéenne, lors qu il s'agit de telles expériences le *test princeps* qui doit les orienter c'est evidemment la recherche de l hematozoaire Si celle ci est positive, le paludisme n est pas guéri que cela déplaise aux théoriciens pour plus ingénieuses qui soient leurs conceptions Et si la recherche de l hématozoaire est négative il faudra une prudente reserve pour formuler des conclusions puisqu'il n'y a pas de malariologiste au moins parmi ceux qui travaillent aux tropiques qui n'ait pas vu qu'il y a des paludéens surtout chroniques, avec d indoubitables symptomes de malaria fievres irregulieres splénomégalie, etc, dont l examen du sang ne décele pas souvent des plasmodies aux plus minutieuses recherches !

Passons donc à exposer mes resultats Dans chaque série on trouvera résumés les divers eléments qui plus détaillément seront publiés dans les *Arquivos da Escola Médico Cirurgica de Nova Goa* dans un prochain numéro

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SERIE I.

Malaria Chronique avec Récidives Pyrétiques

No.	1	2	3	4	5	6
Histoire de la maladié	Fièvre quotidienne pendant 15 jours il y a 2 mois Récidives irrégulières tierces ou quotidiennes	Contracté depuis 8 ans. Type tierces à intervalles irreguliers	Accès quotidienne il y a *15* jours Apyrexie	Malade il y a trois mois accès quo*tidienne pendant* 3 semaines	Malaria tierce *irregulière il y a* quelques mois	Malaria contractée *il y a 1 an Récidives* quotidiennes et tierces irregulières
Symptomes actuels importants	Anémie, teint subictérique Necator Amèbes Ascaris Tricocephale	Nouvel accès typique avec frisson et sueurs	Nouveaux accès Anémie Subictere	Nouvel accès *Lé*gère anémie	Anémie *légère*	Nouveaux accès tierces typiques
Rate	Palpable	Non palpable	Non palpable	Non palpable	Non palpable	Non palpable
Numero total de comprimés de Smalarina	105	120	120	1.0	120	55

Serie I—*suite*

No	1	2	3	4	5	6
Examen parasitologique avant le traitement	Vivax et falcipa rum Schizontes	Vivax Schizontes	Falc parum gamè *tes*	Falciparum gamè *tes (rares)*	Falciparum (gamè *tes Sch rosaces*)	Falciparum (gamè *tes*)
Idem au cours du traite ment.	Falciparum Sch et gamètes	Vivax Sch	Vivax Sch zontes	Falciparum Sch (rares)	Falciparum game tes	
Idem à la fin du traite ment	Falciparum gamè tes	Vivax Sch	Falciparum Schizontes	Falc parum Sch (rares)	Vivax Sch	Falc parum Sch
Pourcentage de Hemoglo b ne avant (Av) au cours (Ac) et à la fin (Af)	Av 40 Ac 39 Af 30	Av 85 Ac 90 Af 90	Av [illegible]0 Ac 5[illegible] Af 6[illegible]	Av 85 Ac 65 Af 75	Av 60 Ac 75 Af 80	Av 93 Ac 90 Af 97
Glob blancs Av Ac et Af	Av 4 500 Ac 4 700 Af 4 600	Av 4 [illegible]00 Ac 5 400 Af 5 [illegible]00	Av 4 400 Ac 7 [illegible]00 Af 8 600	Av 4 [illegible]00 Ac 6 000 Af 7 700	Av 6 000 Ac 9 400 Af 5 600	Av 9 200 Ac 9 300 Af 9 300
Glob rouges Av Ac et Af	Av 2 444 000 Ac 1 750 000 Af 1 [illegible]64 000	Av 4 088 000 Ac 4 120 000 Af 4 123 000	Av 5 [illegible]68 000 Ac [illegible]752 000 Af [illegible]564 000	Av 4 1[illegible]8 000 Ac 3 584 000 Af 4 13[illegible] 000	Av 4 468 000 Ac 5 416 000 Af 3 812 000	Av 4 684 000 Ac 4 292 000 Af 4 960 000

Formule leucocytaire Av Ac et à la fin (Af)

Linfo Pour cent	Av 42 Ac 40 Af 47	Av 57 Ac 55 Af 53	Av 63 Ac 40 Af 40	Av 49 Ac 56 Af 42	Av 57 Ac 40 Af 46	Av. 49 Ac 53 Af 53
Mono „	Av 8 Ac 2 Af 5	Av 0 33 Ac 4 Af 4	Av 3 Ac 7 Af 4	Av 3 Ac 3 Af 4	Av 8 Ac 1 Af 9	Av 1 Ac 6 Af 3
Neutr „	Av 45 Ac 52 Af 46	Av 42 Ac 37 Af 30	Av 31 Ac 37 Af 47	Av 46 Ac 30 Af 42	Av 26 Ac 33 Af 37	Av 43 Ac 34 Af 37
Eosi „	Av 3 Ac 4 Af 1	Av 0 33 Ac 3 Af 2	Av 0 99 Ac 8 Af 8	Av 2 Ac 9 Af 11	Av 6 Ac 16 Af 5	Av 5 Ac 5 Af 4
Baso „	Av 0 21 Ac 0 Af 0 28	Av 0 Ac 0 Af 0	Av 0 24 Ac 0 43 Af 0	Av 0 Ac 0 Af 0	Av 0 Ac 0 Af 0 29	Av 0 Ac 0 63 Af 0 22

SEPIE I—*fin*

Image d'Arneth Av Ac. et Af

No.	1	–	3	4	5	6
II Pour cent	Av 12 Ac 13 Af 15	Av 37 Ac 27 Af 21	Av 5 Ac 10 Af 2	Av 31 Ac 0 Af 3	Av 7 Ac 1 Af 4	Av 15 Ac 5 Af 19
III ,,	Av 34 Ac 46 Af 32	Av 43 Ac 26 Af 32	Av 38 Ac 43 Af 13	Av 43 Ac 12 Af 8	Av 26 Ac 15 Af 34	Av 36 Ac 26 Af 30
IV ,,	Av 38 Ac 23 Af 28	Av 16 Ac 30 Af 23	Av 34 Ac 20 Af 50	Av 13 Ac 50 Af 40	Av 49 Ac 39 Af 37	Av 38 Ac 43 Af 32
V ,,	Av 8 Ac 12 Af 18	Av 3 Ac 13 Af 16	Av 19 Ac 16 Af 23	Av 8 Ac 32 Af 35	Av 15 Ac 30 Af 22	Av 8 Ac 21 Af 14
VI ,,	Av 3 Ac 3 Af 5	Av 0 Ac 2 Af 5	Av 2 Ac. 0 58 Af 6	Av 3 Ac 3 Af 11	Av 1 Ac 12 Af 0 77	Av 1 Ac 4 Af 2

Observations	..	Injection de quinine au cours du traitement pour couper des accès de fièvre	Inj de quinine au cours du traitement	Inj de quinine au début et au cours du traitement	..	Accès de fièvre Inj de quinine au cours du traitement	Inj de quinine au début Le violent accès de fièvre et la repugnance de la malade ou fait arrêter le traitement, au milieu (10 comprimes)
Résultat Final	—	*Nihil* Etat général empire Soumis ultérieurement à quinine et néo salvarsan	Sans acces Résultat parasiticide *nihil* Traitement ultérieur idem	Sans acces Résultat parasiticide *nihil* Traitement ultérieur idem	Sans accès Résultat parasiticide *nihil* Traitement ultérieur idem	Résultat clinique et parasiticide *nihil* Traitement ultérieur idem	Résultat clinique et parasiticide *nihil*

CONCLUSION DE LA SERIE I

Les résultats de la Smalarina chez 6 malades chroniques avec recidives pyrétiques observés jusqu à la fin du traitement peuvent être sommaries de la façon suivante

Effet parasiticide nul 6	100 pour cent
Etat hématologique et général empiré	3—50 „
„ stationnaire	3—50 „
Acces de fièvre en plein cours du traitement	2—33 „
Sans accès de fièvre au cours du traitement	4—66 „

Comme l éxistence ou la non éxistence d un acces febril ne peut pas servir de test revelateur d une infection palustre et ce n est pas en nous basant sur de tels faits d ordre purement clinique que nous pouvons évaluer la valeur anti malarienne d un médicament nous dévons conclure que *dans cette série la Smalarina s est montrée dépourvue de pouvoir parasiticide, au moins jusqu a la fin de ce traitement*

Serie II

Malaria Chronique Avec Recidives Pyretiques Irregulieres et Splenomegalie

(Expériences faites entre Octobre à Mai saison non épidemique a Valpoi, contree Malarienne)

No	1	2	3	4	5
Histoire de la maladie	Malade depuis des annees Accés irreguliers	Malade depuis des annees Accés irreguliers	Malade depuis 4 des mois	Malade depuis des années Derniers accès quotidienns il y a deux mois	Malade depuis 1 an Accès irreguliers
Symptomes actuels importants	Subictere Asthénie Foie hypertrophié	Subictère Asthénie Foie hypertrophié	Anémie Congestion du foie	Foie congestionné	Légère congestion du foie
Rate	A mi distance entre l'ombilic et le rebord costal	Jusqu' à l'ombilic	Deux travers de doigt sous les cotes	Trois travers de doigt sous les cotes.	Deux travers de doigt sous les cotes.
Numero total de comprimés de la Smalarina	136	136	78	130	136
Examen parasitaire avant le traitement.	Falciparum Sch et gamètes	Falciparum Gamètes Vivax Sch	Falciparum Gamètes Vivax Sch	Vivax Sch	Falciparum Sch et gamètes Vivax Sch

Idem au cours du traitement	Falciparum Gamètes et Sch	*Falciparum* Gamètes et Sch Vivax Sch	Falciparum Sch Vivax Sch	Vivax Sch	Falciparum Gamètes, Vivax Sch
Idem à la fin du traitement	Falciparum Sch et gametes.	Falciparum Sch et gamètes	Falciparum Sch et gamètes Vivax Sch	Vivax Sch	Falciparum Gam Vivax Sch
Idem 1 mois après	Falciparum gamètes	Falciparum et Vivax Sch	Falciparum Gam Vivax Sch	Vivax Sch	Falciparum Gam Vivax Sch
Idem 2 mois après	Falciparum Sch et gamètes	Falciparum Gamètes Vivax Sch (51 jours après)	Falciparum Gam *et Sch* Vivax Sch	Vivax Sch (0 jours après)	*Falciparum* Gametes.
Idem 3 mois après	Falciparum gamètes	Falciparum Gamètes. Vivax Sch (61 jours apres)			
Idem 6 *mois* après	Falciparum èt Vivax Sch	Nihil leucocytes mèlanifères	Falciparum Sch (*164 jours apres*)	Vivax Sch (172 jours apres)	Falciparum Sch

SERIE II—*fin*

No		1	2	3	4	5
Formule leucocytaire Av et Après six mois	Linfo Pour cent	Av 39 Ap 53	Av 45 Ap 54	Av 54 Ap 43	Av 48 Ap 45	Av 55 Ap 33
	Mono „	Av 9 Ap 5	Av 3 Ap 1	Av 2 Ap 1 5	Av 4 Ap 5	Av 2 Ap 3
	Neutr „	Av 40 Ap 33	Av 46 Ap 42	Av 34 Ap 38	Av 42 Ap 31	Av 31 Ap 57
	Eosi. „	Av 12 Ap 7	Av 5 Ap 2	Av 9 Ap 15	Av 4 Ap 18	Av 10 Ap 5
	Baso „	Av 0 Ap 0	Av 0 Ap 0 23	Av 0 Ap 0	Av 0 Ap 0	Av 0 Ap 0
Après six mois	II Pour cent	Av 9 Ap 7	Av 6 Ap 33	Av 2 Ap 33	Av 0 Ap 3	Av 2 Ap 4
	III „	Av 25 Ap 23	Av 24 Ap 37	Av 15 Ap 32	Av 18 Ap 16	Av 19 Ap 22

Image d Arneth Av et					
IV „	Av 32 Ap 37	Av 35 Ap 22	Av 40 Ap 21	Av 38 Ap 46	Av 42 Ap 32
V „	Av 27 Ap 23	Av 28 Ap 4	Av 32 Ap 7	Av 29 Ap 26	Av 26 Ap 27
VI „	Av 5 Ap 8	Av 4 Ap 1	Av *10* Ap 3	Av 10 Ap 7	Av 9 Ap 12
Résultat Final	Accès pyrétiques ayant reclamé la quinine et cacodylate Rate molle et légère ment diminuée de volume *Action parasiticide et immunisante Nihil*	Accès *fébrils, 10, 24,* 40, 54 jours après Résultat sur la splénomegalie *nihil* Action parasiticide *nihil,* jusqu à 61 jours après *Action immunisante nihil* (voir leucocytes *mélanifères* et tous les symtômes cliniques)	Actions parasiticide, immunisante, spléno reductrice *nihil* Cliniquement sans accès fébril pendant 70 jours	Actions parasiticide et immunisante *nihil* Spléno re*ductrice insigni*fiante Cliniquement sans accès fébril pendant 70 jours	Actions parasiticide, *immunisante* et spléno reductrice *nihil* Cliniquement sans accès fébril.

Conclusions de la 2nde Serie

Les cinq malades de cette série malariens chroniques avec récidives pyrétiques et splénomégalie, soumis au traitement par la *Smalarina* et observés pendant six *mois apres ce* traitement ont donné les résultats suivants

Action parasiticide nulle 5—100 pour cent

(*N B* —Dans un cas l analyse positive apres 2 mois et négative après 6 *néanmoins les leucocytes mélanifères et les symptomes* cliniques nous authorisent à affirmer la malaria)

Action immunisante nulle	5 — 100	pour cent
„ spléno reductrice nulle	3 — 60	„
„ „ „ insignifiante	2 — 40	„
Améliorations cliniques légères	4 — 80	„
Aucun résultat clinique	1 — 20	„

SERIE III

Malariens Chroniques Splenomegaliques mais Apyretiques depuis 6 4 et 3 mois

(Expériences faites entre Octobre Mai a Valpoi)

No	1	2	3	4
Histoire de la maladie	*Malade depuis des années* Accès irréguliers le dernier il y a trois mois	*Malade depuis des années* Derniers accès il y a quatre mois	*Derniers accès il y a trois* mois	Malade depuis des années. Derniers accès il y a six mois
Symptômes actuels	Foie hypertrophié Asthénie	Foie hypertrophié Asthénie	Anémie	Foie hypertrophié Subictère Asthénie
Rate	Quatre travers de doigt sous les côtes	Fosse illiaque gauche	Légèrement palpable	Un travers de doigt sous l ombilic
Numero total de comprimés de Smalarina	136	136	91	136
Examen parasitaire avant le traitement	Vivax Sch	Falciparum Gam Vivax Sch.	Vivax Sch	Vivax Sch
Idem au cours du traitement	Vivax Sch	Falciparum Gam Vivax Sch	Vivax Sch	
Idem à la fin du traitement	Vivax Sch	Vivax Sch	Vivax Sch	Vivax Sch
Idem 1 mois après	Vivax Sch	Falciparum Sch Vivax Sch.	Vivax Sch (17 jours après)	

Idem 2 mois après		....	Falciparum Sch. Vivax Sch	Vivax Sch (*42 jours après*)	Vivax Sch (62 jours après).
Idem 3 mois après ..		Vivax Sch (*71 jours après*)	...	....	....
Idem 6 mois apres		Falciparum Sch	.	Falciparum et Vivax Sch (*146 jours après*)	*Nihil* Rares leucocytes mélanifères.
Formule leucocytaire Av et Après six mois	Linfo Pour cent .	Av 33 Ap. 44	Av 45 Ap —	Av 56 Ap. 41	Av. — Ap 45
	Mono „	Av 4 Ap 2	Av 3 Ap —	Av 9 Ap 2	Av. — Ap 8
	Neutr „	Av 54 Ap 48	Av 44 Ap —	Av 31 Ap 50	Av — Ap 40
	Eosi „	Av 6 Ap 6	Av 6 Ap —	Av 3 Ap 5	Av — Ap 6
	Baso „	Av 0 Ap 0	Av 0 Ap	Av 0 Ap 0	Av — Ap 0
Image d'Arneth Avant et Après six mois	II Pour cent .	Av 6 Ap 24	Av 12 Ap —	Av 16 Ap 33	Av — Ap 6
	III „	Av 26 Ap 35	Av 46 Ap —	Av 29 Ap 30	Av — Ap 28
	IV „	Av 39 Ap. 29	Av 28 Ap —	Av 37 Ap 28	Av — Ap 12

No		1	2	3	4
Image d Arneth Avent et Apres six mois	V Pour cent	Av 22 Ap 8	Av 11 Ap —	Av 15 Ap 5	Av — Ap 22
	VI	Av 4 Ap 2	Av 1 Ap —	Av 2 Ap 2	Av — Ap 10
Résultats		Action parasiticide et immunisante *nihil* Spléno reductrice insignifiante (à 3 travers de doigt) Cliniquement moins asthénique	Observé à point pendant 60 jours Fièvre après 30 jours Actions parasiticide immunisante spléno reductrice et clinique *nihil*	Enfant de 12 ans Fièvre 8 mois après (saison epidémique) Actions parasiticide immunisante spleno reductrice et clinique *nihil*	Actions parasiticide et immunisante *nihil* Jusqu' à 62 jours Action spléno reductrice insignifianté (jusqu à l ombilic) Asthenie moindre

Conclusions de la Serie III

Les quatre malades splénomégaliques apyrétiques depuis quelques mois soumis au traitement par la S *malarina* et observes pendant six mois ont donné les résultats suivants

Actions parasiticide et immunisante nulle	2—50	pour cent
Idem pendant 60 jours	2—50	„
N a pu etre examiné pendant 6 mois	1—25	
Sans plasmod es à la fin de 6 mois mais avec leucocytes melanifères et signes cliniques le paludisme	1—25	„
Action spléno reductrice nulle	2—50	„
„ insignifiante	2—50	„
Résultat clinique nul	2—50	
„ „ avec légères amél orations	2—50	„

SERIE IV.

Malades du Département de Sanguém (contree malarienne)

(Observations faites entre Décembre—Juin En Juin commence la saison malarienne)

No	1	2	3	4	5	6
Histoire de la maladie	Malade depuis 4 années Acces quotidiens tierces ou irrégulières	Malade depuis 3 ans	Malade depuis des années	Malade depuis des *années*	Malade depuis 5 ans	Malade depuis des années
Symptômes actuels	Acces de 15 à 15 jours le plus souvent	Accès de 4 à 4 jours Foie normal	Asthénie Profonde anémie	Accès de 15 à 15 jours, environ, asthénie	Accès de 6 à 6 mois irregulières	Accès hebdomadaires
Rate	3 doigts de travers sous les cotes	5 doigts de travers sous les cotes depassant la ligne moyenne	5 doigts de travers sous les cotes	Fosse illiaque gauche	Fosse illiaque *gauche*	4 doigts de travers sous les côtes
Numero total de comprimés de Smalarina	136	136	136	136	136	91
Examen parasitaire avant le traitement	Falciparum Sch	Falciparum Sch	Falciparum Sch	Falciparum Sch	Falciparum gamètes	Falciparum Sch
Idem six mois après	Vivax Sch	Falciparum Sch.	Falciparum Sch	Neg	Vivax Sch L mélanifères	Vivax gamètes

Serie IV—*fin*

	No	1	2	3	4	5	6
Formule leucocytaire Av et Ap six mois.	Lymph Pour cent	Av 45 Ap 49	Av 57 Ap 55	Av 50 Ap 47	Av 54 Ap 55	Av 58 Ap 48	Av 41 Ap 44
	Mono "	Av 0·75 Ap 0	Av 4 Ap. 2	Av 1 Ap 0·5	Av 2 Ap 0	Av 0·5 Ap 1	Av 0·75 Ap 2
	Neutr "	Av 48 Ap 47	Av 33 Ap 38	Av 41 Ap 46	Av 40 Ap 41	Av 40 Ap 45	Av 55 Ap 50
	Eosi "	Av 5 Ap 3	Av 3 Ap 3	Av 7 Ap 5	Av 2 Ap 3	Av 1 Ap 4	Av 2 Ap 2
	Baso "	Av 0 Ap 0	Av 0 Ap 0	Av 0·5 Ap 0	Av 0 Ap 0	Av 0 Ap 0	Av 0 Ap 0
d'Arneth Av. et Ap six mois.	II Pour cent	Av 30 Ap 29	Av 25 Ap 21	Av 26 Ap 29	Av 24 Ap 31	Av 42 Ap 20	Av 18 Ap 20
	III "	Av 37 Ap 54	Av 23 Ap 31	Av 46 Ap 38	Av 27 Ap 36	Av 32 Ap 36	Av 36 Ap 40
	IV "	Av 20 Ap 14	Av 30 Ap 31	Av 17 Ap 22	Av 27 Ap 26	Av 15 Ap 26	Av 29 Ap 27
	V "	Av 7 Ap 1	Av 12 Ap 11	Av 7 Ap 6	Av 16 Ap 3	Av 7 Ap 7	Av 13 Ap 6

Image VI	Av 3 Ap 1	Av 6 Ap 4	Av 2 Ap 2	Av 3 Ap 2	Av 2 Ap 4	Av 2 Ap 0
R clinique sur la fièvre	Apyrexie	Trois accès après le traitement	Apyrexie	5 accès fébrils après le traitement	Apyrexie (N B Remarquer que ses accès venaient de 6 à 6 mois même avant le traitement)	Apyrexie
R clinique sur la rate	1 travers de doigt sous les cotes	Insignifiante	2 travers de doigt sous les côtes	Insignifiante	Stationnaire	Stationnaire
R clinique sur l etat general	Amélioré	Légere améliora tion	Sensible améliora tion	Stationnaire	Legere améliora tion	*Sans altération*
Conclusions	Action immuni sante *nihil* Ac tion spléno reduc trice et clinique appréciable	Actions immuni sante spléno re luctrice *nihil* Action clinique insignifiante	Action immuni sante *nihil* Ac tions spléno re ductrice et clini que appréciables	Actions immuni sante spléno reductrice et cli nique *nihil* (On peut l affirmer malgre l examen dernier né at f quant aux plas mod es)	Actions immuni sante spléno reductrice *nihil* Action clinique insignifiante	Actions immuni sante et spleno reductrice *nihil* Action clinique appréciable quant à la fievre

Conclusions de la Serie IV

La six malades de Sanguém soumis au traitement Smalarinique donnent les résultats suivants

Action immunisante nulle	6 — 100 pour cent
Action spléno reductrice appreciable	2 — 33
" " insignifiante	2 — 33 "
nulle	2 — 33 "

SERIE V

Malades du Department de Quepem (contrée malarienne)

(Observations entre *Décembre-Juin*)

No	1	2	3	4	5
Histoire de la maladie	Malade depuis 1 an (enfant de 9 ans)	Malade depuis 4 ans (enfant de 13 ans)	Malade depuis 1 an (enfant de 10 ans)	Malade depuis 3 ans (enfant de 11 ans)	Malade depuis 2 ans Accès irréguliers
Symptômes actuels	Anémie Acces irré*guliers de 15 à 15* de 8 à 8 jours	Accès irrégulèrs	Acces mensuels *chaque accès durant* 8 à 10 jours	Accès irréguliers ou *bi mensuels durant* 8 à 10 jours	Foie hypertrophié *Anémie*
Rate	Légèrement palpable	3 travers de doigt sous les côtes	Un travers de doigt sous les cotes	5 travers de doigt sous les côtes	Deux travers de doigt sous les cotes
Numero total de comprimés de Smalarina	66	91	66	91	136
Examen parasitologique avant le traitement	Falciparum Sch	Falciparum Sch	Vivax Sch et Gamètes	Falciparum Sch	Falciparum Sch
Idem à la fin du traitement	Falciparum Gamètes Vivax Sch	Falciparum Sch		Falciparum Sch	Falciparum Sch.
Idem 6 mois après		Vivax Sch	Vivax Gamètes	*Nihil*	Falciparum et Vivax Sch
6 mois du Linfo Pour cent	Av 40 Af 50 Ap —	Av 48 Af 49 Ap 31	Av 34 Af — Ap 42	Av 59 Af 46 Ap 23	Av 50 Af 50 Ap 45

Formule leucocytaire Av à la fin et après traitement	Mono	Av 0 Af 1 Ap —	Av 2 Af 1 Ap 8	Av 3 Af — Ap 12	Av 4 Af 3 Ap 11	Av 0 25 Af 0 75 Ap 9 60
	Neutr	Av 28 Af 27 Ap —	Av 42 Af 45 Ap 53	Av 53 Af — Ap 36	Av 43 Af 40 Ap 45	Av 47 Af 41 Ap 29
	Eosi	Av 31 Af 0 Ap —	Av 6 Af 4 Ap 7	Av 7 Af — Ap 8	Av 1 Af 8 Ap 15	Av 1 Af 7 Ap 14
	Baso	Av 0 Af 0 Ap —	Av 0 Af 0 Ap 0	Av 0 Af — Ap 0	Av 0 Af 0 Ap 0	Av 0 Af 0 Ap 0 56
Image d Arneth Avant, à la fin et après six mois	II Pour cent	Av 10 Af 14 Ap —	Av 27 Af 24 Ap 15	Av 13 Af — Ap 13	Av 27 Af 22 Ap 9	Av 29 Af 21 Ap 5
	III "	Av 48 Af 43 Ap —	Av 31 Af 32 Ap 34	Av 27 Af — Ap 21	Av 38 Af 37 Ap 48	Av 29 Af 35 Ap 32
	IV "	Av 31 Af 7 Ap —	Av 29 Af 29 Ap 36	Av 37 Af — Ap 61	Av 23 Af 31 Ap 34	Av 26 Af 27 Ap 49
	V	Av 8 Af 10 Ap —	Av 9 Af 11 Ap 10	Av 18 Af — Ap 3	Av 8 Af 7 Ap 7	Av 12 Af 10 Ap 9
	VI	Av 3 Af 3 Ap —	Av 1 Af 2 Ap 2	Av 2 Af — Ap 0	Av 2 Af 1 Ap 0	Av 2 Af 3 Ap 3

SERIE V—*fin.*

No	1	2	3	4	5
Résultat clinique . ..	Les accès continuent	Insignifiante réduction de la rate Foie hypertrophié Acces fébrils	Acces fébrils tierces 3 mois apres	Acces fébrils 3 mois après Rate à trois travers de doigt sous les cotes	Etat général amélioré, rate presque normale Foie réduit Acces palustres 2 et 4 mois après
Conclusions	*N B* Le sang du malade n'a pu etre examiné 6 mois aprés mais à peine à la fin du traitement Action parasiticide à la fin du traitement *nihil* Actions immunisante et clinique *nihil*	Actions parasiticide, immunisante, clinique, *nihil* Action spleno reductrice insignifiante	Action immunisante, spléno reductrice et clinique *nihil.*	Action parasiticide *nihil* spléno reductrice appréciable, clinique insignifiante, immunisante *nihil* (malgre l'examen final parasitaire négatif et en vue d'autres symptomes)	Actions parasiticide et immunisante *nihil* spleno reductrice et clinique appréciables

CONCLUSIONS DE LA SERIE V.

Les cinq malades de cette serie soumis au traitement par la Smalarina ont donne les résultats suivants

Action parasiticide nulle	..	.. 5—100 pour cent
„ immunisante nulle	..	5—100 „
„ spléno reductrice insignifiante	.	1— 20 „
„ „ appreciable	.	2— 40 „
„ „ nulle	.. .	. 2— 40 „
„ clinique insignifiante	. .	. 1— 20 „
„ „ appréciable	.. ..	1— 20 „
„ „ nulle ..	.. ..	.. 3— 60 „

SERIE VI

Malades de la Circonscription de Colem (localité malarienne)

Observations entre Décembre—Juillet (en Juin commence la saison épidémique)

No	1	2	3	4	5
Histoire de la maladie	Malade depuis 2 ans Accès en général irreguliers	Malade depuis 2 ans Accès en général irreguliers	Malade depuis 4 ans Accès de 6 en 6 mois	Malade depuis des années	Malade depuis 8 ans
Symptomes actuels	Derniers accès hebdomadaires	Accès fréquents	Les accès se succèdent souvent pendant un mois	Accès hebdomadaires durant 1 ou 2 jours	Accès irreguliers
Rate	Quatre travers de doigts sous les côtes	Quatre travers de doigts sous les cotes	Quatre travers de doigts sous les côtes	A mi chémin entre le rebord costal et l'ombilic	Cinq travers de doigts sous les cotes
Numero total de comprimes de Smalarina	136	136	136	136	136
Examen parasitaire avant le traitement	Pl Vivax Sch	Falciparum Gamètes	Pas de parasites Leucocytes melanifères	Pas de parasites	Vivax Sch

SERIE VI—*fin*

No		1	2	3	4	5
Idem à la fin du traitement		Pl Vivax Sch	Vivax Sch	Falciparum Sch	Pas de parasites	Vivax Gamètes
Idem 6 mois après			Falciparum Sch	Pas de parasites L mélanifères	Pas de parasites	Vivax Sch
Formule leucocytaire Av à la fin et 6 mois après le traitement	Linfo Pour cent	Av 58 Af 45 Ap —	Av 41 Af 45 Ap 41	Av 31 Af 29 Ap 35	Av 57 Af 42 Ap 59	Av 51 Af 37 Ap 53
	Mono	Av 0·73 Af 0 43 Ap —	Av 2 Af 2 Ap 1	Av 2 Af 2 Ap 4	Av 1 Af 1 Ap 5	Av 1 Af 1 Ap 5
	Neutr „	Av 40 Af 43 Ap —	Av 53 Af 46 Ap 56	Av 55 Af 55 Ap 47	Av 35 Af 49 Ap 32	Av 43 Af 56 Ap 40
	Eosi „	Av 0·48 Af 10 Ap —	Av 2 Af 6 Ap 1	Av 10 Af 13 Ap 12	Av 6 Af 5 Ap 3	Av 5 Af 4 Ap 1
	Baso „	Av 0 Af 0 Ap —	Av 0 Af 0 Ap 0	Av 0 Af 0 Ap 0	Av 0 Af 0 Ap 0	Av 0 Af 0 Ap 6
après le	II Pour cent	Av 29 Af 33 Ap —	Av [illegible] Af 28 Ap 12	Av 16 Af 27 Ap 5	Av 21 Af 28 Ap 12	Av 23 Af 30 Ap 15

Image d Arneth Av à la fin et 6 mois tra tement	III „	Av 30 Af 34 Ap —	Av 35 Af 43 Ap 21	Av 33 Af 33 Ap 34	Av 37 Af 33 Ap 41	Av 41 Af 27 Ap 25
	IV	Av 24 Af 25 Ap —	Av 25 Af 22 Ap 26	Av 8 Af 6 Ap 19	Av 28 Af 23 Ap 33	Av 23 Af 31 Ap 33
	V	Av 10 Af 5 Ap —	Av 13 Af 4 Ap 30	Av 36 Af 29 Ap 28	Av 7 Af 10 Ap 17	Av 6 Af 5 Ap 22
	VI „	Av 4 Af 2 Ap —	Av 2 Af 0 58 Ap 8	Av 2 Af 3 Ap 12	Av 4 Af 4 Ap 7	Av 4 Af 4 Ap 4
Résultat Clinique		Nihil après 20 jours	Nihil	Amélioration Rate à deux travers de doigt sous les cotes.	*Nihil* Accès fébrils 3 et 4 mois après Rate augmentee (fosse iliaque gauche)	*Nihil* Accès febrils Rate augmentée (fosse iliaque gauche)
Conclusions		Le malade n a pu etre examiné après 6 mois Actions parasiticide et spléno reductrice *nihil* Action clinique insignifiente	Actions parasiticide immunisante spléno reductrice et clinique *nihil*	Action parasiticide *nihil* à la fin du tra tement Actions spléno reductrice et clinique appréciables Action *immunisante ?*	Actions immunisante spleno reductrice et clinique *nihil* Action parasiticide ? *N B* Remarquer que dans aucune analyse on n a trouvé des parasites malgré des signes évidents de malaria !	*Actions* parasiticide immunisante, spléno reductrice et clinique *nihil*

Conclusions de la Serie VI

Les cinq malades de la Série VI soumis au traitement par la Smalarina ont donné les résultats suivants

Action parasiticide nulle	4—80 pour cent
N B On n a pu connaître cette action dans 1 cas	
Action immunisante nulle	4—80
douteuse	1—20
spléno reductrice nulle	4—80 „
appreciable	1—20
clinique nulle	4—80
appreciable	1—20

Note —Toutes les analyses parasitaires ont éte faites par moi même et dans les cas de Valpoi les analyses intermediaires par le Dr Braz de Sa Toutes les analyses hematologiques par mes éleves Vernencar et Naique L observation clinique chez les malades des provinces appartient à mes délegues de santé Braz de Sa (Valpoi) J F Afonso (Quepem) J M Gracias (Sanguem) et A J Vas (Colem qui ont vivement collabore dans cette enquete

L ingestion des comprimés de Smalarina n a pas éte suivie d aucun résultat facheux quelques nausees et vertiges ont rapidement cédé à la suspension temporaire du méd cament et à l administration d'un purgatif

Analysant les resultats d ensemble chez les 31 malades qui font l object de ce rapport on voit

Quant a l'action parasiticide

Nulle	25—80 pour cent
N ont pas montre des plasmodies à la fin de l observation mais avaient d autres signes evidents du paludisme	4—12 8
N avaient pas de parasites mais ceux ci n ont pas aussi ete trouves avant le traitement	2—6 4 „

Quant a l action immunisante

Nulle	21—67 pour cent
Douteuse	2—6 4 „
Nulle pendant 60 jours	2—6 4 „

Quant a l action spleno reductrice

Nulle	13—42 pour cent
Insignifiante	7—22 „
Appreciable	5—16
N a pu etre examine	1—3 2

Quant a l action clinique

Nulle	16—51 ? pour cent
Insignifiante	12—38 „
Appreciable	3—9 6

Conclusion Finale —Dans nos essais therapeutiques chez des malades choisis en diverses contrees malariennes et observes jusqu à six mois apres le traitement la Smalarina Cremonese s est montree dépourvue de valeur soit parasiticide soit immunisante et nous ne saurions pas conseiller cette drogue comme arme anti malarienne soit à titre curatif soit à titre prophylactique

ON THE CHRONICITY OF MALARIA IN FORMOSA

BY

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THE systematic control work of malaria in Formosa commenced far back in 1911, having continued up to the present time There are about 70 local malaria preventive stations at present to the care of which are placed 116 districts where about 1 700,000 populations are treated every year The principal measures of preventive work are the regular blood examination, once a month, of residents living in those districts and the administration of quinine to the carriers found on that occasion All peoples (persons above certain age are exempted in some districts) must be examined being prescribed by the law, and nobody must object Adding to this the peoples and authorities of the districts are obliged to endeavour to destroy Anopheline mosquitoes and their breeding places

In spite of continuous endeavour however there are many places where the desirable results can not still be obtained Only in the cities, due to the completion of the sewage construction the Anopheline mosquitoes have markedly diminished and the malaria infection almost never occurs while there are some places which remain uncultivated owing to the condition of the configuration

Thus, the annual percentage of the carriers average throughout the island for some years is as follows —

Year	1917	1918	1919	1920	1921	1922	1923	1924	1925	1926
Percentage	3.13	2.13	2.45	1.96	1.94	2.60	2.50	2.92	2.43	2.13

This result fluctuating according to the area concerned, cannot be an exact measure to decide the effect of the preventive work hitherto done The result in each district, however, can be useful to estimate the true effect of the work In most places it appears to have resulted in the decrease of the carriers, although there are some places where even an inclination of those to increase is seen

Notwithstanding I am of the opinion that this apparent decrease of the carriers is not due to a true disappearance of the parasites from the blood, but due to the chronicity of malaria in which the parasites become very few, appearing irregularly in the peripheral blood

It is noteworthy that in Formosa many carriers remain uncured the patients newly infested becoming carriers and thus, the carriers, both latent and active, may increase year by year As a peculiar fact the visitors of the preventive station to take medicine seem to be almost of same

People are often seen who have showed the parasites on almost every occasion of monthly blood examination, although they have taken the medicine on every occasion From their condition, I believe, these cases are suffering from relapses as well as reinfections There are many persons in such a condition throughout the island This must be an important problem both from the malaria epidemiological point and from the social sanitary point of view

What is the reason for such a phenomenon? What is the measure against it?

According to my opinion this fact depends on the overlooking of the chronic patients especially in the latent stage, and the failure of the treatment, at least for those suffering from very chronic and inveterate malaria

The method of treatment used at present is as follows —

Dosis pro die adult — 0 8 grm of quinine hydrochloride
children (below 15) — 0 1 to 0 6 grm of quinine hydrochloride or 0 2 to 0 8 grm of euchinin (according to age)

TABLE I

Selection from the protocols showing frequent infections or appearances of parasites

1 *Results obtained at Hayashida*

Name	sex & age	Findings of Blood Examinations																								
		1925										1926												1927		
		III	IV	V	VI	VII	VIII	IX	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	I	II	III
R A	♂ 44			○						△			△				△			△			△			△
R T	♀ 42					△		△														△				
R S	♀ 22		△			△○		△		○		○					△					○				
T.M	♀ 12		○		○		○	○								△			△	○			□	□		
R K	♀ 15				△		○	○			○				○					△		○		△		
R B	♀ 7				△	△	△				△				△○						△		△	△		
R E	♀ 4																		○	△○		△○	△		○	○
S S	♂ 26																		○		△	△○	△	△	△	
R E	♀ 20																		△	△	△				△	○
I S	♂ 29						○	○									○		○		○	○	○		△	
I H	♂ 5					△○											△○		○		○	○		△	△	
M Y	♂ 14		○		□		○					○					○			□		○				

B *Results obtained at Hozan*

Name	sex & age	Findings of Blood Examinations																																		
		1922									1923												1924												192	
		IV	V	VI	VII	VIII	IX	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	I	II
.	♂ 6	○	△	○					○													△						△	△						△	
.	♂ 16																○		△			○	△						△					△		
.	♀ 4																△		○		△	△	△													△
.	♀ 8			△								△														○				△						□
.	♀ 5		○												○			△					△					△								
.	♂ 5																			△			○		△				△		○		△			
.	♀ 3																					△	○	△	○		○		○			△				
.	♀ 6						△	△	□	△		□														△			△		○					△
.	♀ 6	△	○	○		○									○		○		△		○	△				△										△

△ designates tertian, □ quartan, and ○ tropical infection respectively

Formula of administration —

6 days' administration followed by 3 days' pause

3 days' administration followed by 3 days' pause, repeated four times

Thus a total of 14 4 grms of quinine are taken during 30 days by the adult This dosis seems to be insufficient for the chronic patients In addition to this the want of supervision of the administration more or less lessens the effect Further more, the recent inclination of knowledge on the mode of action of quinine is towards accepting the theory that the action should collaborate with the function of certain internal organs In most chronic patients it may be taken for granted that such organs are to some extent retarded in function If it is so in such cases seen in Formosa the effect of quinine may be incomplete, at least if used in the usual manner From the points mentioned above a more proper method of the treatment should be devised to help Formosa to free from the chronicity of malaria

The other important problem must be how to detect the latent infection The control work will not succeed without solving this problem

For blood examination the thin film method is mostly employed at present but the effect may be more prominent by using the thick film method instead It may not be so difficult to devise a convenient way of applying the thick film method to conditions in the field

It is reasonable that the rate of the finding of parasites varies with the number of blood examinations made during a certain period Table II shows how more effective two examinations of blood in a month is than one

TABLE II

Results of blood examinations at the village Hayashida where about 600 people were examined twice a month

RESULTS	POSITIVE CASES FOUND IN EACH MONTH							
Blood Examination 1937	January	February	March	April	May	June	July	August
The 1st time	50	25	8	11	8	11	13	10
The 2nd time (new findings)	33	21	20	9	13	21	24	22
TOTAL	83	46	28	20	21	32	37	32

As seen in the above table the second examination made 15 days after the first have added many new findings It may be definitely said that the more times the blood is examined the greater is the effect Actually, however, frequent

examinations of a large number of the residents every month is impossible. Each malaria preventive station bears an average population of 2,306 at present. If so, it is necessary to determine the most suitable time and opportunity for the blood examinations which should not be too frequent but yet which should give a better result.

In this connection the first thing to be known is the behaviour of the Plasmodium. It is well known that in chronic cases the parasites do not always appear in the peripheral blood. To know the reasons for the fluctuation of the parasites if present would give valuable material for the determination of suitable times and opportunities for blood examination.

On this subject some observations have been carried out by the writer and the result shows that the behaviour of the peripheral parasites falls into three categories:—

(1) Cases in which the parasites are almost always seen.
(2) Cases in which very few parasites appear and only occasionally.
(3) Cases in which a large number of the parasites appear concentrated within certain consecutive days with or without clinical symptoms.

From these facts it is concluded that no rule on the behaviour of the parasites common to all cases is present; therefore no particularly suitable time and opportunity for blood examination exists. Thus the problem of the times and opportunities for regular blood examination remains unsolved.

Another method attempted by the writer for the diagnosis of latent malaria is that the persons suspected of latent infection are provisionally chosen by means of other signs among those who do not show parasites in the ordinary blood examination. The urobilinogen reaction, though not special to malaria, seems to be applicable for this purpose. This reaction is known as occurring in almost all cases of acute malaria, while in chronic cases it does occur but not constantly. Recent examination by the writer and his co-workers observing in a large number of chronic cases has pointed out many interesting facts. In tropical regions the group of people amongst whom the urobilinogen reaction is more prominent show a higher parasite or spleen rate and a higher combined parasite and spleen rate. The leucocytic picture of most people showing a positive reaction is similar to that in the carriers. It is of especial interest that the urobilinogen rate in a place is markedly decreased after two months' quininization of the entire residents. Furthermore we can detect latent infections in 41.6 per cent of the people who had formerly discharged urobilinogen without parasites in the peripheral blood.

From these facts we can suggest that the increase of urobilinogen discharge in tropical regions is usually due to malaria, be it latent or active. From this point of view people showing a positive urobilinogen reaction must be suspected of having an infection even when the parasite is not detected.

I have two plans as to the further treatment of such persons who have been isolated by means of the urobilinogen reaction. The one is the method of making

persons, showing strong positive reaction take the medicine unconditionally This method may call for some discussion but in a tropical region the probability of infection among such persons is very high and deeming them as latent carriers may be not far from the truth The other method for such persons is to continue further concentrated blood observation Such concentrated examination is, however, hard to put into practice every month The regular blood examinations, therefore, must be reduced to two or four times a year We working in a certain region, have used this method successfully

Notwithstanding we have hitherto been taught by experience that control work against the malaria may never succeed without considering the destruction of Anopheline mosquitoes because infection does not become absolutely extinct even with most effective methods known for the patient and the carrier Parallelism of work on both lines is always needed The territorial condition in Formosa however compels the control work for Anopheline mosquitoes to remain far behind that for the patients and carriers This is one of the fundamental reasons why the control work of malaria in Formosa cannot prominently succeed In addition to this the important problem regarding the breeding places of Anopheles is that the rice fields increase year by year There are some parts where the malaria epidemic is apparently due to the increase in the rice fields It is very necessary that systematic investigation into the relation between malaria endemics or epidemics and rice fields should be carried out and that suitable measures for that problem be discovered

In conclusion the malaria in Formosa has become chronic and it cannot be dealt with by common methods It is our pride that systematic preventive work has been continued for many years commencing far back but I deplore that the work has not markedly succeeded even though it has been prevented by the natural circumstances At present we stand at the point where more effective methods of mass diagnosis and treatment must be devised and where more suitable measures for the destruction of Anopheline mosquitoes require to be established

QUELQUES MOYENS BIOLOGIQUES DE DIAGNOSTIC DU PALUDISME LATENT.

PAR

TRUONG DINH-TRI

ET

TRINH HUU-LOI,

Communication faite par les bons soins de Monsieur le Docteur Jourdran, Directeur du Service de Santé du Tonkin

Il nous est arrivé plusieurs fois d'observer chez nos malades annamites traités pour asthénie nerveuse des accès de paludisme franc au cours d'une cure par des injections strychno-cacodyliques

Ces accès de paludisme ont revêtu dans la généralité des cas un caractère solennel de fièvre à trois stades frisson, chaleur et sueur Ils apparaissent ordinairement vers le 4è ou 5è jour du traitement chez des malades en état d'apyrexie et qui n'ont présenté depuis de longues années aucun mouvement fébrile

Ayant négligé les premiers cas chez lesquels des petites doses de quinine avaient vite raison, nous avons pensé dans la suite à prélever le sang sur lames en plein accès de fièvre et à pratiquer nous mêmes l'analyse microscopique Dans tous ces examens, nous avons trouvé des hématozoaires, forme jeune

L'idée nous est donc venue de chercher à dépister les cas de paludisme latent par l'administration par voie hypodermique de strychnine ; d'autre part la lecture des résultats des recherches faites dans le même sens avec d'autre produit comme l'adrénaline et publiées par A Dazzi dans 'Il Policlinico' (Sezione Pratica) Rome, Tome XXVI, fascicule 48, du 30 Novembre 1919, nous a suggéré l'idée d'étendre nos champs d'investigation avec l'emploi de l'adrénaline

Nous avons laissé de côté l'emploi de l'ergotine, de l'hypophysine, etc

Ce sont les résultats de ces recherches faites sur une vingtaine de cas que nous exposons dans ce travail

Que ce soit avec la strychnine ou l'adrénaline, l'action qui détermine la diffusion de l'hématozoaire dans le torrent circulatoire périphérique serait identique Cette action se traduit par une réduction temporaire du volume de la rate hypertrophiée ou par la simple contraction des fibres cellules contractiles du tissu splénique Dans l'un et dans l'autre cas, il y a une véritable 'expression de la rate'

Comme nous l'avons dit plus haut, la strychnine ne produit son effet qu'au bout de 4 ou 5 jours d'expérience Cela tient vraisemblablement d'une part à la dose faible que nous avons employée et d autre part a la contraction qui ne se produit dans les organes à fibres musculaires lisses que tardivement, c'est a dire bien apres celle des muscles de la vie animale La dose journalière que nous avons adoptée a eté invariablement chez l adulte de un milligramme administree par voie hypodermique

Bien entendu, nous avons élimine, dans nos recherches, les sujets qui n'ont pas d'antécédents paludéens averés les excités nerveux, les épileptiques et les vieux arterio scléreux et hypertendus Dans bon nombre de cas, nous avons profité des necessités thérapeutiques pour poursuivre nos investigations

Avec la strychnine, nous avons pu experimenter sur onze malades a antécé dents paludéens manifestes dont trois ayant présenté de la mégalosplénie d'un volume moyen, la rate n ayant dans aucun cas depassé plus de trois travers de doigt des fausses cotes Parmi ces onze cas experimentés, nous n'avons trouve des hématozoaires que sur 5 cas seulement Nous relatons ci dessous les quelques observations les plus typiques

I Homme de 28 ans metayer a compté 20 mois de séjour à Cho Go (Yen The) pays réputé paludeen et malsain Il y a contracté du paludisme qui a été soigné avec de la quinine et des arse nicaux Retonrné dans le delta depuis plus de trois ans il est venu en 1923 me consulter à Phu Lang Thuong Depuis son retour il ne ressentit plus de fièvre Etat géneral bon anémie légère hyper trophie splenique dont la matité ne dépasse pas les fausses cotes de deux travers de doigt Anorexie, état asthénique assez prononce attribue par le malade à du surmenage physique récent J ai prescrit des injections quotidiennes d une ampoule de

Sulfate de strychnine	1 mg
Cacodylate de soude	5 cgms
Serum physiologique	5 c cs

Au bout de 2 ou 3 jours l etat s était amélioré le malade declarait recouvrer l appetit, il se fatiguait moins et dormait beaucoup

Au 5e jour, deux heures apres la piqure le malade fut pris subitement de frisson tres violent qui fit place une demi heure apres à une stade de chaleur Appele d urgence à son chevet je lui pratiquai, après une prise de sang prealable sur deux lames une injection de Quinoforme de un gramme Au bout de 4 heures l accès de fièvre cessa avec une transpiration profuse Les deux lames de sang colorées au Bleu de Méthylene boraté ont présenté des formes jeunes d hématozoaires à type tierce

Les jours suivants, j administrai au malade en plus de la piqûre strychno cacodylique habituelle un gramme de sulfate de quinine per os et repete pendant une semaine

II Homme de 34 ans secrétaire ayant fait 7 ans auparavant 18 mois de sejour à Hà Giang pays répute insalubre A eu peu de temps après ce sejour des accès de fièvre traités à la Quinine et au Diemenal Depuis son retour dans le delta c est à dire depuis 7 ans n a plus d accès de fièvre Il vient me consulter en Janvier 1924 à Hung Yen pour asthénie et surmenage Même traitement que pour le malade de l observation N°1 injections strychno cacodylique

Au 4e jour quelques heures apres la piqure acces solennel et typique de fièvre paludeenne Le sang preleve sur lames et coloré au Giemsa a presente les formes jeunes du type tierce

III Homme de 47 ans opiomane ayant séjourné il y a quinze ans à Lao Kay region tres insalubre Peu après son retour a eu du paludisme à forme intermittente traité à la quinine Depuis plus de douze ans n a pas présenté de fievre

Il vient nous consulter en juin de cette année pour asthenie amaigrissement et anorexie Traitement un verre à madère de Vin de Quinquina au moment de chacun des principaux repas (extrait mou de quinquina 2 grs glycérine 3 grs et Vin de Lunel) injection hypodermique quotidienne

le sulfate de Strychnine de un milligramme Au 6e jour grand accès de fièvre palustre à 3 stades Le sang prélevé et coloré au Giemsa a présenté la forme schizonte de la tierce Traitement quinique institué dans la suite Guérison

Nous avons essayé l adrénaline sur 10 cas, dans aucun cas nous n'avons observé des acces de fievre franche Chez trois sujets expérimentés nous avons constaté dans la journee meme un léger mouvement fébrile (de 37°5 à 38°) que nous attribuons plutot à l'action hyperthermisante de l adrénaline Parmi ces trois sujets ayant eu ce léger mouvement thermique, un seul a presenté des hémato zoaires dans le sang Nous avons employé la dose uniforme de un milligramme soit un centim cube de la solution au 1 000e

L injection a été faite strictement dans le tissu cellulaire lache sous cutane, et partant de la conception theorique qui admet la destruction facile du produit quand il est injecté dans le derme ou dans les muscles nous avons apporté un soin méticuleux dans la pratique de nos injections

Dans quatre cas nous avons observé des parasites dans le sang tous de forme jeune a un intervalle variant de 6 a 10 heures après l injection

Chez aucun de ces malades nous n avons observé les hematozoaires les jours qui suivirent l injection d ailleurs nous nous étions contentes d une injection unique

Conclusions

L emploi de l Adrénaline et de la Strychnine peut rendre des services tres utiles pour le diagnostic du Paludisme latent

La Strychnine a l avantage de provoquer des acces de fievre franche, elle a aussi celui d un maniement facile lorsqu elle est employée à des doses raisonnables et si elle a des contre indications l Adrénaline a aussi les siennes peut être plus nombreuses

Mais son action est tres retardée et c est seulement à ce point de vue que l Adrénaline l emporte

RESOLUTIONS ON MALARIA

DISCUSSION

SATURDAY DEC 10TH 10 A M TO 12 NOON

THE CHAIRMAN [*Col S P James, I M S* (retd) (G Britain)] called on Sir Malcolm Watson to read the draft of the *first* resolution

Sir Malcolm Watson (F M S) The Malaria Section of the Seventh Congress of the Far Eastern Association of Tropical Medicine are aware of many instances of a great increase in the incidence of malaria caused by the facilities given to mosquito reproduction by engineering works either during construction or afterwards due to the different conditions brought about This Congress is of the opinion that plan for railways canals harbours and all similar engineering works likely to affect the conditions producing malaria should be submitted to the proper public health authorities and their sanitary engineers before being sanctioned by Governments

THE CHAIRMAN spoke in favour of the resolution

Lieut Col C A Gill I M S (Punjab) Considered that the resolution would be of great value to health officers

Dr R Row (Bombay) Agreed with the resolution but considered that the human factor, in the form of the labour force employed on such works should be included in the resolution

Bt Col S R Christophers, I M S (B India) Agreed and suggested that the word 'schemes' should replace the word 'plans'

Mr Senior White (B India) Considered that the resolution should be more specific and that in the case of railways the chief medical officer should be consulted

Dr J W Scharff (Straits Settlements) Moved that the word 'plans' be retained He considered that it was important that actual plans should be submitted

Bt Col S R Christophers, I M S (B India) Thought, with reference to Mr Senior White's suggestion that it would be a mistake to be too specific, if the resolution were to become more detailed it would require more thought He considered it equally valuable in its present form

THE CHAIRMAN again read the resolution which in the presence of 88 members, was carried with one dissentient vote

THE CHAIRMAN then called on Sir Malcolm Watson to read the draft of the *second* resolution

Sir Malcolm Watson (F M S) As it has been represented that differences of opinion regarding the best method of controlling malaria sometimes cause doubt in the public mind and so may hamper the progress of anti malarial work, this Congress takes the present opportunity to emphasize the fact that there is no single method of malaria control applicable to all conditions and all countries

Nevertheless, they consider that for towns, mines, plantations, large public works and similar aggregations of people, the control of the breeding places of the malaria carrying species of mosquito is a method which should be employed whatever other

anti-malarial measures are put into force Whenever possible this control should be effected by permanent works which eliminate entirely the sources of mosquito breeding

For wide rural areas, specially those with scanty, poverty-stricken populations, the first step in the control of malaria is adequate research, so that the conditions present may be ascertained and the best methods of control under the particular circumstances ascertained as a result of such research Methods of prevention may here be of great variety and include drainage, flooding jungle clearing, jungle preservation, bonification, the promotion of agriculture, improvement of housing and the general economic condition education, etc, of the people The systematic killing of infected adult mosquitoes, screening, the use of quinine and a host of special methods have each also to be considered in their proper application

The Congress desires to stress the need not only of thoroughly trained malaria research officers, but of expert malarial engineers in whichever type of malaria prevention is at stake

Major J A Sinton, I M S (B India) Objected to the use of the word 'quinine' as it would tie down medical officers to the use of one drug He suggested the words 'anti-malarial drugs' in place of the word 'quinine'

Sir Malcolm Watson (F M S) Accepted the proposed alteration

Dr S K Ganguli (Bengal) In passing the resolution on malarial control by preventive measures, suggested that the conditions of (1) 'dying rivers' of Bengal, and (2) 'occluded drainage due to faulty railway construction' in Bengal should be taken into consideration and research on these two vital points be undertaken, and that, *amongst other things, they should form part of the resolution*

Bt-Col C A Gill, I M S (Punjab) Considered the resolution an excellent one It was by way of being a compromise, but it was a compromise which entirely satisfied all parties

Dr D P Williams (Assam) Proposed that the words 'entomological research' be specially stressed amongst the 'research workers'

Bt-Col S R Christophers, I M S (B India) Opposed the inclusion of these words

Dr S L Sarkar (Bengal) Suggested that the 'oiling of tanks' should be included amongst the measures suggested

Dr S K Ganguli (Bengal) Thought that the duties of malarial or anti malarial engineers should be defined

Sir Malcolm Watson (F M S) Pointed out that the resolution only mentioned certain measures as examples and thought that it was unnecessary to include all possible anti malarial measures He considered that the resolution in the form that he had just read it was very satisfactory

THE CHAIRMAN Asked the meeting to give their opinion on the inclusion of the words 'entomological research'

Five members were in favour of these words *being included*

Ninety members were against their inclusion

The amendment was thus defeated.

The resolution, as last read, was put to the meeting and was carried unanimously (103 members present)

RESOLUTIONS ON MALARIA

The RESOLUTIONS ON MALARIA in their final form, as passed at the Business Meeting of the Association were as follows —

RESOLUTION I

The Malaria Section of the Seventh Congress of the Far Eastern Association of Tropical Medicine are aware of many instances of a great increase in the incidence of malaria caused by the facilities given to mosquito reproduction by engineering works, either during construction or afterwards due to the different conditions brought about This Congress is of the opinion that plans for railways, canals, harbours and all similar engineering works likely to affect the conditions producing malaria should be submitted to the proper public health authorities and their sanitary engineers before being sanctioned by Governments

RESOLUTION II

As it has been represented that differences of opinion regarding the best method of controlling malaria sometimes cause doubt in the public mind and so may hamper the progress of anti malarial work this Congress takes the present opportunity to emphasize the fact that there is no single method of malaria control applicable to all conditions and all countries

Nevertheless, they consider that for towns, mines, plantations, large public works and similar aggregations of people the control of the breeding-places of the malaria-carrying species of mosquito is a method which should be employed whatever other anti malarial measures are put into force Whenever possible this control should be effected by permanent works which eliminate entirely the sources of mosquito breeding

For wide rural areas, specially those with scanty, poverty stricken populations, the first step in the control of malaria is adequate research, so that the conditions present may be ascertained and the best methods of control under the particular circumstances ascertained as a result of such research Methods of prevention may here be of great variety and include drainage flooding jungle clearing, jungle preservation, bonification, the promotion of agriculture, improvement of housing and the general economic condition, education, etc , of the people The systematic killing of infected adult mosquitoes, screening, the use of anti malarial drugs and a host of special methods have each also to be considered in their proper application

The Congress desires to stress the need not only of thoroughly trained malaria research officers, but of expert malarial engineers in whichever type of malaria prevention is at stake

VOLUME II.

INDEX OF AUTHORS.

The numbers refer to the pages in the Table of Contents The names and figures pr ted in Itali s are references to the remarks made by Authors in the course of the D s ussions